FINAL REPORT

Investigation of Chemical Reactivity, Mass Recovery and Biological Activity During Thermal Treatment of DNAPL Source Zones

SERDP Project ER-1419

OCTOBER 2009

Kurt D. Pennell
Tufts University

Frank E. Löffler
Jed Costanza
Kelly E. Fletcher
Nivedhya S. Ramaswamy
Gretell Otano
John Callaghan
Georgia Institute of Technology

This document has been approved for public release.

SERDP

DoD EPA DOE

Strategic Environmental Research and Development Program

Report Documentation Page

1. REPORT DATE **OCT 2009**	2. REPORT TYPE **N/A**	3. DATES COVERED

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Investigation of Chemical Reactivity, Mass Recovery and Biological Activity During Thermal Treatment of DNAPL Source Zones	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Tufts University**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release, distribution unlimited.

13. SUPPLEMENTARY NOTES
The original document contains color images.

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT **unclassified**	b. ABSTRACT **unclassified**	c. THIS PAGE **unclassified**	**SAR**	**181**	

TABLE OF CONTENTS

LIST OF TABLES

Acronyms and Abbrevations

AFB	Air Force Base
Ag^+	silver ion
ASTM	American Society for Testing and Materials
bvcA	vinyl chloride reductive dehalogenese gene present in *Dhc*. Strain BAV1
BDI	Bio-Dechlor Inoculum™
Bgs	below ground surface
BLAST	basic local alignment search tool
^oC	degrees Celsius
Ca^{2+}	calcium ion
$CaMg(CO_3)_2$	dolomite
$CaSO_4$	calcium sulfate
CCl_4	carbon tetrachloride
C_2Cl_2	dichloroacetylene
C_4Cl_6	hexachlorobutadiene
C_6Cl_6	hexachlorobenzene
CES	Current Environmental Solutions, Inc.
$CHCl_3$	chloroform
$CHOO^-$	formate
CH_3COO^-	acetate
CH_4	methane
Cl^-	chloride ion
Cl_2HC_2OOH	dichloroacetic acid
cm	centimeter
Co^{2+}	cobalt ion
CO	carbon monoxide
CO_2	carbon dioxide
$COCl_2$	phosgene
COH_3COO^-	glycolate
$COOHCOO^-$	oxalate
CROW	Contained Recovery of Oily Wastes™
CT	carbon tetrachloride
Cu	copper
cis-DCE	*cis*-1,2-dichlororethene
trans-DCE	*trans*-1,2-dichloroethene
DCB	dithionite-citrate-bicarbonate
DCM	dichloromethane
Dhc	*Dehalococoides*
DI	deionized
DNA	deoxyribonucleic acid
DNAPL	dense non-aqueous phase liquid
DO	dissolved oxygen

DOD	Department of Defense
DUS	dynamic underground stripping
ED	electron donor
EGDY	East Gate Disposal Yard
EPICS	equilibrium partitioning in closed systems
EPR	enhanced passive remediation
ERH	electrical resistive heating
Fe	iron
Fe^{2+}	ferrous iron
Fe^{3+}	ferric iron
FeOOH	iron oxide mineral
FeS_2	pyrite
Fe_2O_3	hematite
FID	flame ionization detector
FX	flux meter
g	gram
GC	gas chromatograph
GWERD	Ground Water and Ecosystem Restoration Research
h	hour
H	hydrogen
H^+	hydrogen ion
H_2	hydrogen gas
H_2CO_3	carbonic acid
HFK	Helgeson-Kirkham-Flowers
HP	Hewlett-Packard
HPLC	high pressure liquid chromatograph
HPO	hydrous pyrolysis oxidation
hr	hour
IC	ion chromatograph
ISCO	*in-situ* chemical oxidation
ISCR	*in-situ* chemical reduction
ISTD	in-situ thermal desorption
kg	kilogram
LB	Luria Bertani
M	moles
mg	milligrams
mL	milliliter
mm	millimeter
mM	millimoles
Mg^{+2}	magnesium ion
Mn	manganese
MNA	monitored natural attenuation
MS	mass spectrometer
MSD	mass selective detector
nd	not determined
N_2	nitrogen gas

NAVFAC	Naval Facilities Engineering Command
$NaHCO_3$	sodium bicarbonate
Na_2CO_3	sodium carbonate
$Na_2S_2O_8$	sodium persulfate
NFESC	Naval Facilities Engineering Service Center
NIH	National Institutes of Health
NIST	National Institute of Standards and Technology
NOM	natural organic matter
OD	outside diameter
OW	a mixed, methanogenic, PCE-to-ethene-dechlorinating
PAH	polycyclic aromatic hydrocarbon
PCB	polychlorinated biphenyl
PCE	tetrachloroethene
PCR	polymerase chain reaction
PEEK	polyether ether ketone
pH	logarithm of hydrogen ion activity in solution
PNNL	Pacific Northwest National Laboratories
ppb	parts per billion
ppm	parts per million
ppmv	parts per million, volume basis
PMW	process monitoring well
PTFE	polytetrafluoroethene
PV	pore volume
RNA	ribonucleic acid
rRNA	ribosomal ribonucleic acid
Redox	reduction-oxidation
r^2	correlation coefficient
rpm	revolutions per minute
RSD	relative standard deviation
s	second
S^{2-}	sulfide ion
SER	steam enhanced remediation
SERDP	Strategic Environmental Research Development Program
SO_4^{2-}	sulfate
SPH	Six-Phase Heating™
SRS	Savannah River Site
tceA	trichloroethene reductive dehalogenese gene
TCA	1,1,1-trichloroethane
TCD	thermal conductivity detector
TCE	trichloroethene
USEPA	US Environmental Protection Agency
USACE	US Army Corps of Engineers
UZA	ultra zero grade air
vcrA	vinyl chloride reductive dehalogenese gene
VC	vinyl chloride
VOA	volatile organic analysis

XRD	X-ray diffraction

Symbols

A	pre-exponential factor in Arrhenius equation
C	concentration
Δ	change
E_a	activation energy
I	ionic strength
k	first-order reaction rate constant
K_d	soil-water distribution coefficient
H	dimensionless Henry's Law constant
M	mass
μ	micro
Ω	ohms
P_o	saturated vapor pressure
R	ideal gas law constant
t	time
$t_{1/2}$	reaction half-life
T	temperature
V	volume

Subscripts

$DCEs$	dichloroethenes
g	gas
l	liquid
o	initial or saturated
s	solid or soil
sat	saturation
TCE	trichloroethene
VC	vinyl chloride
w	water

EXECUTIVE SUMMARY

Chlorinated organic solvents, such as trichloroethene (TCE) and tetrachloroethene (PCE), are common pollutants at military and industrial facilities, and represent one of the most difficult contamination scenarios facing site managers. Such sites typically contain a source zone, consisting of dense non-aqueous phase liquid (DNAPL) existing as entrapped ganglia and/or as high-saturation pools residing above low permeability media. Due to the combination of low aqueous solubility and mass transfer limitations, DNAPL-contaminated aquifers serve as long-term sources of groundwater contamination, which may persist for decades or even centuries. To achieve substantial DNAPL mass reduction within acceptable time frames, several in situ remediation technologies have been developed, including chemical oxidation, thermal treatment, air sparging, co-solvent flushing, and surfactant flushing. Of these technologies, thermal treatment provides two distinct advantages: (a) no chemical agents are introduced into the subsurface, and (b) the potential to efficiently treat heterogeneous porous media. In situ thermal treatment technologies, such as Electrical Resistance Heating (ERH), are capable of removing substantial chlorinated solvent mass from the subsurface and may cause in situ degradation of contaminants.

Although elevated temperature has been shown to enhance rates of PCE and TCE reactivity, considerable uncertainty exists when attempting to estimate the fraction of contaminant mass that is degraded during thermal treatment. The goal of our research was to advance the understanding of abiotic and biotic reactions that promote in situ contaminant destruction during thermal treatment. Greater understanding of these reactions may lead to reductions in the duration and temperature required for thermal treatment, along with development of polishing methods to treat residual contamination. The overall objective of this project was to investigate fundamental physical, chemical and biological processes governing thermal remediation of DNAPL source zones. Specific objectives of the project included: a) quantifying relationships between subsurface temperature, physical-chemical properties of chlorinated ethenes and sorption-desorption parameters, b) determining the rate and extent of chlorinated ethenes destruction in contaminated field samples as a function of temperature, c) assessing the effect of temperature on isolates, PCE-to-ethene consortia, and dechlorinating bacteria native to a contaminated field site, d) evaluate the destruction and recovery of chloroethenes during laboratory-scale thermal treatment of field contaminated soil.

E.1. RESEARCH APPROACH

This project was designed to provide a more complete and fundamental understanding of the effects of elevated temperature on physical, chemical and biological processes contributing to the transformation of chlorinated ethenes. The project was structured around four tasks: (1) Contaminant Phase Distribution; (2) Chemical Reactivity and Byproduct Formation, (3) Microbial Reductive Dechlorination; (4) Thermal Treatment Performance Evaluation. To investigate specific mechanisms and quantify causal relationships, batch and flow-through reactor experiments were performed in two- and three-phase (gas-liquid, solid-liquid, solid-liquid-gas) systems containing PCE or TCE over a temperature range of 25 to 800 $^{\circ}$C. In addition, laboratory-scale electrical resistive heating (ERH) studies were preformed to assess contaminant mass recovery and chemical reactivity in DNAPL-contaminated field soils. In these

studies, ERH was chosen as the thermal treatment method since it was used at each of the four sites from which field samples were obtained.

E.2. KEY FINDINGS

Results of this work demonstrate that although TCE and PCE may undergo abiotic transformation and degradation at temperatures typically encountered during thermal treatment (i.e., 25 to 120 °C), the observed rates were slow, yielding disappearance half-lives that ranged from 40 to 7,000 days. In addition, microbial reductive dechlorination of TCE and PCE was negligible at temperatures above approximately 40 °C. Thus, chlorinated solvent recovery during thermal treatment is likely to be dominated by enhanced mass transfer from the solid and liquid phases, while in situ transformation processes provide only minimal contributions to TCE and PCE treatment under most conditions. Although PCE and TCE mass transfer from field-contaminated soils to groundwater correlated to increasing temperature, substantial contaminants levels persisted in fine-grained soils even after heating at 95 ^{o}C for up to 185 days. Thus, even after removal of the dissolved-phase and non-aqueous phase liquid (NAPL) chlorinated ethenes, a substantial fraction of contaminant mass can remain associated with the solid phase, particularly in low-permeability soils with high clay and silt contents. This slowly desorbing fraction of contaminant mass may require prolonged heating combined with vapor and liquid extraction to achieve remediation goals, and could result in the rebound of groundwater contaminant concentrations once thermal treatment ceases. One promising strategy to address such residual contamination is the coupling of thermal remediation efforts, either in series or in parallel, with compatible treatment technologies including bioremediation and oxidation-reduction processes.

E.2.1. Rates of Abiotic TCE and PCE Degradation are Relatively Slow at Temperatures Less than 120 ^{o}C

Experiments completed as part of this project were designed to determine the rate of PCE and TCE degradation in soil and groundwater samples collected from four field sites undergoing thermal treatment including: Camelot Cleaners Superfund Site, Great Lakes Naval Training Center, East Gate Disposal Yard (EDGY) in Fort Lewis, and the Pemaco Superfund Site (Table E-1).

Results obtained from a large matrix of heated ampule experiments indicate that the half-life for PCE degradation in Camelot soil and groundwater was greater than 7,000 days at 95 °C, while the relatively small amount of TCE present was transformed to *cis*-1,2-dichloroethene (*cis*-DCE) with a half-life of 26 days. No degradation of PCE, TCE, or *cis*-DCE was observed in ampules containing soil and groundwater from the Great Lakes site. In ampules containing Fort Lewis field samples, TCE was transformed to *cis*-DCE at 25 °C by microbial activity and was degraded abiotically at 95 °C with a degradation half-life of 1.6 to 1.9 years. However, the fraction of contaminant transformed by the thermal processes was relatively small, accounting for less than 5-10% of initial PCE or TCE mass. These findings indicate that in situ transformation processes occurring during thermal remediation are likely to provide only minimal contributions to TCE and PCE treatment at these thermal remediation field sites. However, faster degradation rates and greater mass conversion may be achieved in the presence of reactive minerals or conditions at contaminated sites. For example, in ampules containing Ottawa sand and 1% (wt.) goethite, abiotic degradation of TCE at 120 °C yielded a half-life of 102 days over the initial 22 days of incubation.

Table E-1. Field sites undergoing thermal treatment from which pre-thermal treatment soil samples were collected.

Site Name	Location	Soil Type	Contaminant
Camelot Cleaners Superfund Site	West Fargo, ND	Clay	PCE
East Gate Disposal Yard, DNAPL Area #3	Fort Lewis, WA	Glacial Outwash (Gravel to Clay)	TCE
Great Lakes Naval Training Center, Site 22	Great Lakes, IL	Silty Clay	PCE
Pemaco Superfund Site	Maywood, CA	Silty Sand	TCE

E.2.2. Microbial Reductive Dechlorination of Native and Laboratory Pure Cultures and Consortia Ceases at Temperatures above 40 °C, but can be Recovered with Post-Treatment Bioaugmentation and Biostimulation.

The effect of temperature on biotic reductive dechlorination of chlorinated ethenes was investigated using two PCE-to-ethene mixed consortia, Bio-Dechlor Inoculum™ (BDI) and OW. These cultures were amended with PCE and excess electron donor and incubated at temperatures ranging from 24 to 45 °C. Complete reductive dechlorination to ethene occurred only in cultures incubated between 24 and 30 °C. At incubation temperatures above 30 °C, either vinyl chloride was the final end product (35 °C) or no dechlorination of PCE occurred (45 °C). These findings provide evidence that, at temperatures greater than 40 °C, complete reductive dechlorination of PCE or TCE to ethene is unlikely to occur.

Additionally, microcosms were constructed with soils and groundwater from the Great Lakes and Fort Lewis sites to assess the potential for enhanced biotic reductive dechlorination at field sites. The microcosms were incubated at temperatures of 24, 35, 50, 70, and 95 °C. No reductive dechlorination occurred in any of the microcosms constructed with Great Lakes soils. In microcosms constructed with Fort Lewis soil, reductive dechlorination of TCE to *cis*-DCE occurred only in microcosms incubated at 24 °C. TCE-to-*cis*-DCE dechlorination occurred in the Fort Lewis microcosms incubated at 35 °C following the addition of an electron donor (lactate), suggesting that lack of suitable electron donor(s) in microcosms incubated at 35 °C limited reductive dechlorination. After 4 months of incubation, the microcosms were cooled to 24 °C and no reductive dechlorination occurred during the cool-down period. To assess the feasibility of post-thermal treatment bioaugmentation and determine if thermal treatment provides beneficial conditions for subsequent enhanced microbial dechlorination via bioaugmentation, the microcosms were inoculated with consortium OW. Reductive dechlorination of PCE to *cis*-DCE and vinyl chloride occurred in all microcosms, but electron donor addition (i.e., biostimulation) was required to achieve complete reductive dechlorination of PCE to ethene.

No reductive dechlorination occurred at temperatures above 40 ºC in experiments with the PCE-to-ethene dechlorinating consortia BDI and OW, which were enriched under mesophilic conditions. However, these experiments do not rule out the existence of microbes capable of PCE reductive dechlorination at elevated temperatures. Similarly, dechlorinating bacteria native to Fort Lewis soil did not dechlorinate PCE at temperatures above 24 ºC unless electron donor was supplied, at which time PCE dechlorination occurred at 35 ºC. Although the coupling of bioaugmentation following thermal treatment requires further investigation, results obtained with the Great Lakes and Fort Lewis soils suggest that both biostimulation and bioaugmentation will be required to achieve complete and rapid dechlorination of PCE and TCE to ethene.

E.2.3. Formation of Byproducts (e.g., CO_2) During Thermal Treatment of Field Soils may Result From Degradation of Soil Organic Matter Rather than Contaminant Destruction.

Potential breakdown products of chlorinated ethenes have been reported in samples obtained from contaminated sites undergoing thermal treatment. This observation has led to speculation that chlorinated ethenes undergo transformation reactions during thermal treatment, resulting in enhanced in situ contaminant destruction. To investigate these claims, ampule experiments were conducted to quantify byproduct formation from contaminated soil and groundwater collected from Camelot Cleaners, Great Lakes, and Fort Lewis field sites (see Table E-2). The ampules were heated to temperatures between 25 and 100 °C and samples were collected from the gas, aqueous, and solid phases of each ampule during incubation and analyzed for a wide variety of compounds, including anions (organic acids, chloride) and volatile organic compounds (chlorinated ethenes, CO, CO_2, ethene).

Consistent with anecdotal field reports from sites undergoing thermal treatment, compounds other than the parent contaminants (TCE and PCE) were observed in ampules containing soil and groundwater following incubation at elevated temperatures. These compounds were also observed after incubating uncontaminated soil samples, which suggests that compounds detected do not represent TCE and PCE degradation products. For example, PCE-contaminated soil collected from the Camelot Cleaners site yielded 1-butene upon heating, but this compound was also detected at similar concentrations after incubating PCE-free Camelot soil. Thus, at elevated temperatures, 1-butene represented a breakdown product of Camelot soil, rather than a product of PCE degradation.

A major breakdown product of chlorinated ethenes undergoing thermal degradation is CO_2, which was observed after heating all of the field soils. Although CO_2 has been shown to be an oxidation product of chlorinated ethenes in well-controlled laboratory experiments (Knauss et al., 1999; Costanza et al., 2005), the amount of CO_2 detected in the incubated field soils was orders-of-magnitude greater than the amount of CO_2 that could be theoretically contributed from the initial amount of contaminant mass (PCE or TCE) present. Therefore, the detection of elevated CO_2 levels during thermal treatment may indicate the destruction of the contaminants, but is more likely an indication of soil breakdown processes. Based on these findings, changes in CO_2 levels should not be used to infer in-situ contaminant destruction or to estimate the mass of contaminant destroyed in-situ.

Table E-2. Compounds detected in gas-phase during incubation.

Incubation temperature	25°C	50°C	70°C	95°C
Camelot PCE (parent)	CO_2	CO_2 TCE	CO_2 TCE	CO_2, CO, c/tDCE, TCE, 1-butene, benzene, furan
Fort Lewis TCE (parent) *cis*-DCE (parent)	CO_2 CH_4	CO_2, CO, CH_4, acetylene, ethene, ethane	NA	CO_2, CO, CH_4, acetylene, ethene, ethane
Great Lakes PCE (parent) TCE (parent) *cis*-DCE (parent)	CO_2, TCA	CO_2	CO_2	CO_2, CO, CH_4, acetylene, ethene, ethane, 1-butene
Pemaco TCE (parent)	CO, CO_2	CO, CO_2	CO, CO_2	CO, CO_2, 1-butene, furan

In none of the experiments completed at temperatures between 50 and 100 °C were TCE and PCE concentrations observed to decrease with a corresponding increase in potential breakdown products. Rather, TCE and PCE aqueous phase concentrations tended to increase with temperature due to contaminant desorption from the solid phase, and remained relatively constant over incubation periods of up to 200 days. Although some TCE and PCE may have undergone degradation at elevated temperatures, these findings show that the rate of PCE and TCE degradation under these conditions is very slow, and is masked by soil decomposition processes. In order to conclusively demonstrate degradation of chlorinated ethenes at elevated temperatures, well-controlled studies must be performed with both contaminated and uncontaminated soil and groundwater samples. Such studies should be performed in sealed glass ampules or metal enclosures because TCE and PCE were both found to rapidly diffuse through the polymer septa of volatile organic analysis (VOA) vials at temperatures above 50 °C.

E.2.4. Although Thermal Treatment can Increase Mass Transfer of TCE and PCE from Contaminated Soils to Groundwater, a Substantial Fraction of Contaminant Mass may Persist in Fine-Grained Soils.

Increasing the temperature of soil has been shown to decrease the sorption of chlorinated ethenes by soil, implying that thermal treatment will greatly enhance mass transfer and recovery of chlorinated ethenes from the subsurface. To evaluate these processes, the masses of TCE and PCE remaining in soil after incubation were determined by repeated methanol extractions. Although an increase in the amount of TCE and PCE present in the aqueous phase of heated ampules was observed, a substantial fraction of mass remained associated with the solid phase. The mass remaining differed for the various soil types, with soils from Camelot Cleaners and Great Lakes sites, both of which contain relatively high clay and silt content compared to Fort Lewis soil, retaining more PCE/TCE. As an example, the Great Lakes soil contained 641 mg/kg

of PCE after incubating at 95 °C for 185 days, as compared to Fort Lewis where there were only 2.9 mg/kg of TCE after 95.5 days at 95 °C. In addition, complete mass recovery of PCE from Great Lakes soils required six sequential methanol extractions, demonstrating that PCE was strongly retained by this soil even after prolonged heating. This observation is also supported by the analysis of soil samples collected from the Great Lakes field site three months after thermal treatment ceased, which contained up to 84 mg/kg of PCE even though groundwater PCE concentrations were below the detection limit of 5 μg/L. These findings demonstrate that subsurface heating will increase contaminant mass transfer from soil to groundwater; however, prolonged heating and water flushing, or some other polishing step, may be required to achieve complete removal of chlorinated ethenes from the solid phase. The persistence of solids-associated contaminants is most likely to be of concern in fine-textured soils, and may lead to contaminant rebound in post-treatment groundwater samples.

E.2.5. Use of Reactive Amendments during Thermal Treatment Leads to Enhancement in Rates of Contaminant Recovery from Slowly-Desorbing Soil Fractions.

Rather than relying on intrinsic reactivity, amendments that promote in-situ chemical oxidation (ISCO) or reduction (ISCR) could be used at sites undergoing thermal treatment to increase the rate of in-situ contaminant destruction. The combination of thermal treatment with ISCO or ISCR could take advantage of heating to increase the rate of contaminant desorption and in-situ destruction, respectively, to minimize above ground waste handling and disposal. A series of experiments was conducted with sodium persulfate, a water soluble and heat activated oxidant, to assess the potential benefits of combining ERH and ISCO. While only 4.8% of the PCE recovered from ERH treatment of Great Lakes soils was oxidized by sodium persulfate, the total mass of PCE recovered increased by 41% compared to treatment with ERH alone. Thus, treatment with sodium persulfate during ERH may enhance the rate of PCE recovery from the slowly desorbing fraction of Great Lakes soil. This effect was attributed to soil breakdown processes and corresponding release of PCE, and provided further evidence of the potential importance of soil properties and soil-bound contaminant mass on the effectiveness of thermal remediation technologies.

CHAPTER 1

INTRODUCTION

1.1 PROJECT JUSTIFICATION AND SCOPE

Chlorinated organic solvents, such as tetrachloroethene (PCE) and trichloroethene (TCE), are common pollutants at military and industrial facilities, and represent one of the most difficult contamination scenarios facing site managers. The contamination at a typical chlorinated solvent site can be divided into two main regions; a highly concentrated *source zone*, and a lower concentration zone that includes a *dissolved solute plume* and *sorbed-phase contaminant*. The source zone usually contains free liquid solvent, commonly referred to as dense non-aqueous phase liquid (DNAPL), existing as entrapped ganglia and as high-saturation pools residing above low permeability lenses or confining layers. Due to the combination of the low aqueous solubility of chlorinated solvents and mass transfer limitations, the DNAPL-contaminated source zone serves as the long-term source of groundwater contamination that may persist for decades or even centuries. To achieve substantial DNAPL mass reduction within an acceptable time frame, several in situ remediation technologies have been developed including chemical oxidation, thermal treatment, air sparging, co-solvent flushing, and surfactant flushing (e.g., Christ et al., 2005; NRC, 2003; Stroo et al, 2003).

Of the *in-situ* remediation technologies introduced above, thermal treatment provides two distinct advantages;

- No chemical agents are introduced into the subsurface

- The potential to efficiently treat heterogeneous and/or low-permeability porous media.

The term "thermal treatment" encompasses a number of technologies designed to deliver thermal energy (heat) to the subsurface, including hot water injection, steam injection, conductive heating, resistive heating, and electromagnetic heating. When heat is introduced to the subsurface, physical-chemical properties of DNAPL can be altered substantially, which in turn governs contaminant distribution between the solid (soil), liquid (water) and gas (if present) phases. For example, the saturated vapor pressure (P_o) of TCE and PCE increases 30-50 times, from 0.098 to 2.92 atm and from 0.024 to 1.20 atm, respectively, as the temperature is increased from 25 to 100 ^{o}C. Similarly, the dimensionless Henry's Law constant (H) for TCE increases approximately 15-20 times from 25 to 100 ^{o}C (Heron et al., 1998a). As a result, even modest heating (50-70°C) can substantially alter contaminant phase distribution, with a large fraction of mass ($> 50\%$) likely to exist in the gas phase of low organic carbon content (i.e., $\leq 0.1\%$ wt) soils and aquifer materials.
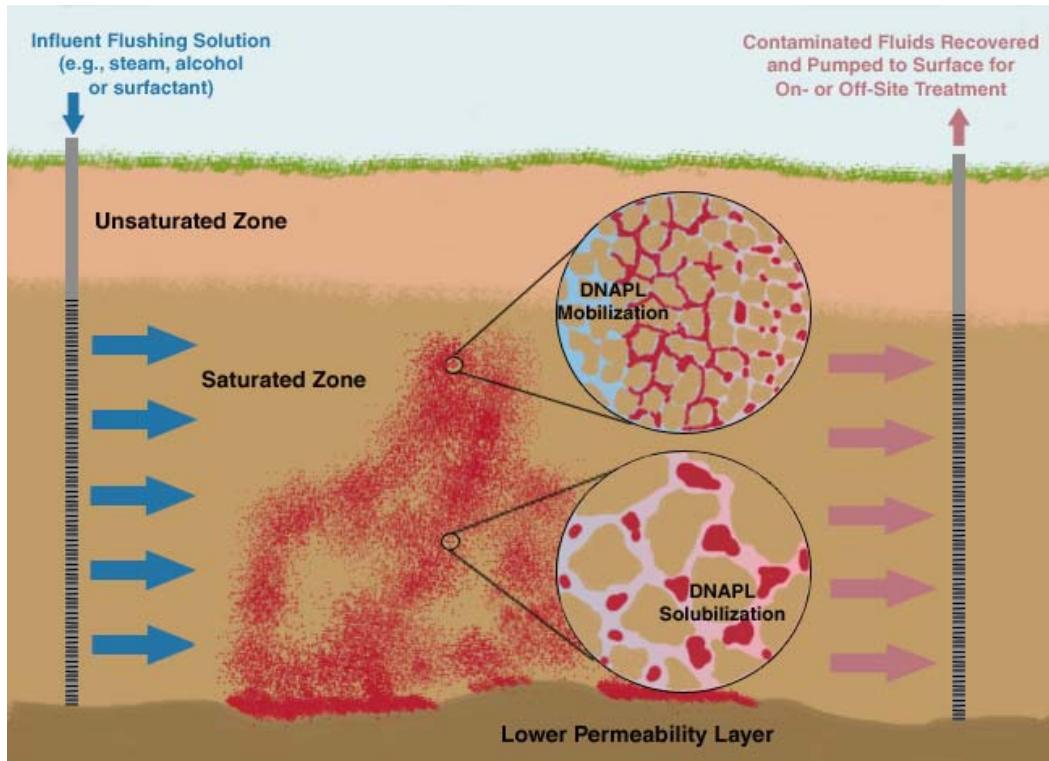
Figure 1-1. Schematic diagram of a DNAPL source zone in a shallow, relatively homogeneous, unconfined aquifer. Insets illustrate two in situ flushing recovery mechanisms: top inset shows mobilization of free product DNAPL due to interfacial tension reduction, lower inset shows enhanced solubility of entrapped DNAPL mass via dissolution or solubilization) (Christ et al., 2005, with permission from *Environmental Health Perspectives*).

Chlorinated ethenes, including PCE, TCE, *cis*-1,2-dichlororethene (*cis*-DCE), *trans*-1,2-dichloroethene (*trans*-DCE), and vinyl chloride (VC), are thought to be relatively stable in subsurface environments. Half-lives of greater than 100,000 years have been estimated for the disappearance of PCE, TCE and *cis*-DCE in water at 25 ^{o}C (Jeffers et al., 1989). Increasing the temperature of contaminated subsurface environments is believed to increase the rate of chlorinated ethene degradation. In the presence of dissolved oxygen, the complete conversion of dissolved-phase TCE to carbon dioxide (CO_2) and chloride ions (Cl^-) has been claimed to occur during thermal treatment, a process referred to as hydrous pyrolysis oxidation (HPO) (Knauss et al., 1999). Although dissolved-phase TCE is known to degraded within heated reactors, the reaction products are thought to include dichloroacetic acid (Cl_2HC_2OOH), as well as CO_2 and Cl^-. In addition, heating of gas-phase TCE in air to temperatures above 200 ^{o}C is known to result in the formation of phosgene ($COCl_2$) and fully-chlorinated compounds, including PCE and carbon tetrachloride (CCl_4) (Ryan et al., 1996). Furthermore, the presence of minerals and organic matter in the subsurface may facilitate or catalyze the generation of byproducts other than those found in homogeneous, aqueous- and gas-phase systems. However, very little is

known about the identity of the transformation products or the parameters that control thermal transformation process.

It is now recognized that even successful source zone treatment technologies will not completely remove all separate-phase DNAPL mass, and are likely to increase the mobility and distribution of the residual mass in the short term, as evidenced by increased aqueous phase concentrations (Udell and Itamura, 1998). Thus, a key issue facing site managers is whether or not monitored natural attenuation (MNA) or enhanced passive remediation (EPR) can be relied upon to control plume development and migration following aggressive treatment of DNAPL source zones. To date, the potential effects of thermal treatment on microbial community structure and reductive dechlorination have only rarely been studied, and have primarily focused on nonhalogenated organic contaminants. Following steam treatment of a JP-5 jet fuel site at Naval Air Station Lemoore, microbes were widely distributed even in zones that reached temperatures of 90 $^{\circ}$C (Udell et al., 1994; Newmark and Aines, 1995). In laboratory studies, mesophilic bacteria were detected after steam treatment of contaminated field soils, but microbial activity occurred only in soils that were allowed to cool gradually (Richardson et al., 2002). Although microbial characterization of subsurface environments following thermal treatment is limited, monitoring data suggest that microbial rebound may occur after field-scale steam injection (Udell et al., 1994; Newmark and Aines, 1995, Krauter et al., 2005). During steam or hot water injection, however, it is likely that the subsurface will become aerobic, especially when air is co-injected to promote chemical oxidation or control downward migration of free product. In contrast, field measurements of reduction-oxidation (redox) potential during electrical resistive heating were reported to be compatible with reductive dechlorination (Beyke et al., 2000).

1.2. PROJECT OBJECTIVE

The overall objective of this project was to provide a more fundamental and comprehensive understanding of the effects of in situ thermal treatments on chlorinated ethene (PCE and TCE) phase distribution, chemical reactivity, and microbial activity.

1.3. TECHNICAL APPROACH

The research activities of the project were structured around the following four tasks: (1) Contaminant Phase Distribution; (2) Chemical Reactivity and Byproduct Formation, (3) Microbial Reductive Dechlorination; and (4) Thermal Treatment Performance Evaluation. The research scope and general experimental approach used in each task are described below.

1.3.1. Task 1. Contaminant Phase Distribution

Task 1 involved the collection of soil and groundwater samples from four field sites undergoing thermal remediation and the determination of equilibrium and kinetic desorption parameters for the field soils. Additionally, temperature-dependent Henry's Law constants and aqueous solubilities were determined for PCE- and TCE-water systems over a temperature range of 20 to 90 $^{\circ}$C.

1.3.2. Task 2. Chemical Reactivity and Byproduct Formation

Task 2 was designed to determine the rate and extent of chlorinated ethene degradation in flame-sealed glass ampoules containing contaminated field soils and groundwater subject to various incubation temperatures. A separate matrix of experiments was undertaken to evaluate the ability of heat-activated sodium persulfate to increase the rate of chlorinated ethene degradation in the field soils. In addition, flow through reactor experiments were conducted to identify and quantify combustion products formed when a gas stream containing PCE or TCE was passed through a quartz tube heated to fixed temperatures ranging from 20 to 800 $^{\circ}$C.

1.3.3. Task 3. Microbial Reductive Dechlorination

Task 3 focused on the response and resilience of dechlorinating pure cultures and consortia to increases in temperature. Experiments were performed to evaluate the effects of thermal treatments on electron donor availability and the viability of species that compete with dechlorinating organisms. The ability of dechlorinating consortia to treat a dissolved-phase PCE plume emanating from an electrical resistive heating (ERH) treatment zone was also demonstrated under this task.

1.3.4. Task 4. Thermal Treatment Performance Evaluation

Laboratory-scale experiments were conducted in an aquifer cell to assess the effect of ERH treatment on contaminant mass recovery and chemical reactivity. These experiments also involved the use of sodium persulfate to cause the in situ destruction of chlorinated ethenes during ERH treatment.

1.4. REPORT ORGANIZATION

This report provides a comprehensive description of experimental systems, analytical methods and corresponding results obtained from the research performed during this project. The organization of chapters within this report is consistent with SERDP final technical report guidance and includes the following sections:

1. Introduction
2. Background
3. Materials and Methods
4. Results and Discussion
5. Conclusions and Implications

Within the Materials and Methods and Results and Discussion sections, specific activities and research findings are organized under each of the tasks described above.

CHAPTER 2

BACKGROUND INFORMATION

2.1 INTRODUCTION

During the past twenty years a number of in situ remediation technologies have been developed to treat chlorinated solvent source zones, including chemical oxidation, thermal treatment, air sparging, co-solvent flushing and surfactant flushing. Thermal treatment offers two distinct advantages when compared to other source zone remediation technologies: (a) no chemical agents are introduced into the subsurface, and (b) the potential to efficiently treat heterogeneous porous media. Despite the ability thermal remediation technologies, such as electrical resistive heating, to effectively remove contaminant mass from the subsurface as demonstrated in several pilot- and full-scale field applications (U.S. EPA, 2004), the costs associated with thermal treatment can be quite high. For example, thermal treatment costs for relatively small field sites are often reported to exceed $1,000,000 (FRTR, 2002; NFESC, 2007). Thus, there is a clear need to optimize thermal treatment systems to improve mass destruction and recovery, and to potentially enhance in situ biotic degradation of contaminants, either during or following thermal treatment. Such optimization approaches could not only render thermal treatment more effective from a mass destruction point of view, but would also make the technology more competitive from a cost perspective. Unfortunately, only limited data are available regarding the phase distribution, chemical reactivity, and reductive dechlorination of chloroethenes at elevated temperatures in subsurface environments.

This section of the report is intended to provide a conceptual description of the most common thermal treatment technologies, compare and contrast their advantages and disadvantages, and review the basic principles of abiotic and biotic chlorinated ethene reactivity at elevated temperatures.

2.2. THERMAL TREATMENT TECHNOLOGIES

At a fundamental level, heat can be defined as the vibration of molecules in a substance. The transfer or delivery of heat (thermal energy) to a contaminated soil matrix can be achieved through several mechanisms, including convection, conduction, radiation. In Table 2-1, the five in situ thermal remediation technologies introduced above are compared in terms of operational factors, including heat transfer mode, upper temperature range, and phase recovery. More detailed descriptions of each technology, as well as potential limitations and knowledge gaps, are discussed below.

Convective heating involves the transfer of heat via a mobile fluid (e.g., liquid or gas), similar to the coolant in an engine. Processes based on convective heat transfer, including steam and hot water injection, exhibit upper temperature limits in the range of 100-120 °C (Udell, 1997). A hot water injection technique, referred to as Contained Recovery of Oily Wastes (CROW™), was developed in the late 1980's to displace and recover NAPLs from the subsurface (U.S. Patent No. 4,884,460, Johnson and Sudduth, 1989). The CROW™ technology has been implemented at two Superfund sites: Columbia Gas Plant Superfund site in Columbia, PA and the Brodhead Creek Superfund Site in Stroudsburg, PA. Although substantial contaminant mass recovery was achieved at the Brodhead site (~1000 gallons of coal tar), the

CROW™ technology was unable to reduce the contaminant mass to residual (immobile) levels, and failed to achieve long-term remediation goals (U.S. EPA, 2000).

The injection of steam as the mobile convective heating fluid has been successfully implemented at both the pilot- and full-scale, and has received considerable attention in the scientific literature (e.g., Davis, 1997; Udell, 1997; She and Sleep, 1999). Subsurface steam injection was initially investigated by petroleum engineers as a means to enhance oil recovery through viscosity reduction and distillation (e.g., Volek and Pryor, 1972). In the 1980's the technology was adapted for treatment of NAPL-contaminant source zones, and often incorporates aggressive vapor and dual phase extraction systems to capture contaminants in the gas, liquid water and NAPL phases (U.S. Patent No. 5,018,576, Udell et al., 1991). The resulting process is often referred to as Steam Enhanced Remediation (SER)/Dynamic Underground Stripping (DUS). The technology has been tested at several field sites containing DNAPL, including the Savannah River Site located in Aiken, SC and the Portsmouth Gaseous Diffusion Plant located in Portsmouth, OH. However, delivery of mobile, heated fluids to a contaminant source zone are subject to preferential flow and density over-ride effects (Udell, 1997). Such phenomena may be especially problematic when contaminants exist in either low-permeability zones and as DNAPLs, which tend to migrate downward through aquifer formations upon mobilization. To address the latter issue, several approaches have been proposed to minimize the formation of condensed DNAPL banks, including the creation of lower steam boundary (Gerdes et al., 1998) and co-injection of air with steam (Kaslusky and Udell, 2002; Schmidt et al., 2002).

Conductive heat transfer occurs through the direct contact of two objects, where molecular vibrations of one object cause the molecules of an adjacent object to vibrate. Due to the proximity of molecules in each phase, solids exhibit the highest heat conductivity, followed by liquids and gases. The technology was originally developed to enhance desorption of contaminants from the subsurface in conjunction with gas phase extraction, coined in-situ thermal desorption (ISTD) (U.S. Patent No. 5,190,405, Vinegar et al., 1993). In practice, heat is delivered to the contaminated soil matrix using either steel well casings or surface "thermal blankets", and vacuum extraction systems are used to recover volatilized/vaporized contaminants through a centrally-located extraction well or a network of combined heater-extraction wells (e.g., Iben et al., 1996; Conley and Lonie, 1998; Stegemeier and Vinegar, 2001). Relatively high in situ temperatures (e.g., 500-800 °C) can be achieved with conductive heating, and thus the technology is frequently used to treat source zones containing semi-volatile contaminants, such as polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs). At these elevated temperatures it has been suggested that PCBs and other chlorinated compounds undergo complete oxidative destruction (e.g., Stegemeier and Vinegar, 2001). However, PAH growth and formation of dibenzofurans from chlorophenols has been widely reported in combustion reactors at temperatures ranging from 500 to 600 °C (e.g., Yang et al., 1998; Mulholland et al., 2000).

Electrical resistive heating (ERH, a.k.a. "Joule" or "Ohmic" heating) involves the introduction of an electric current into the subsurface, from which heat is generated as electrons pass through the soil matrix, which acts as a resistor (U.S. Patent No. 4,957,393, Buelt and Oma, 1990). More specifically, the conduction of electrical current occurs primarily through soil pore water (Robain et al., 2003), and thus, soil resistivity varies strongly with water content, soil mineralogy and electrolyte content. Soil resistivity values can vary from greater than 109 Ω/cm for dry soils to approximately 102 Ω/cm for soils containing dissolved salts at 15-20% (wt.) water content (Kalinski and Kelly, 1993). In effect, dry soils serve as very efficient electrical

insulators, which can result in poor heat propagation and necessitating the replenishment of water at the electrode to avoid melting (Heron et al., 1998b). In field applications, electrical current has been delivered in either a three- or six-phase mode (U.S. Patent No. 5,330,291, Heath et al., 1994). The latter process, commonly referred to as Six-Phase Heating™ (SPH) has undergone pilot-scale (single array) testing at a number of DNAPL contaminated sites, including the Savannah River Site, Area M, and Cape Canaveral, Launch Complex 34. Although Six-Phase Heating™ provides for efficient heating of the subsurface in relatively small, circular electrode arrays, three-phase heating is much less complex and safer to implement at larger scales.
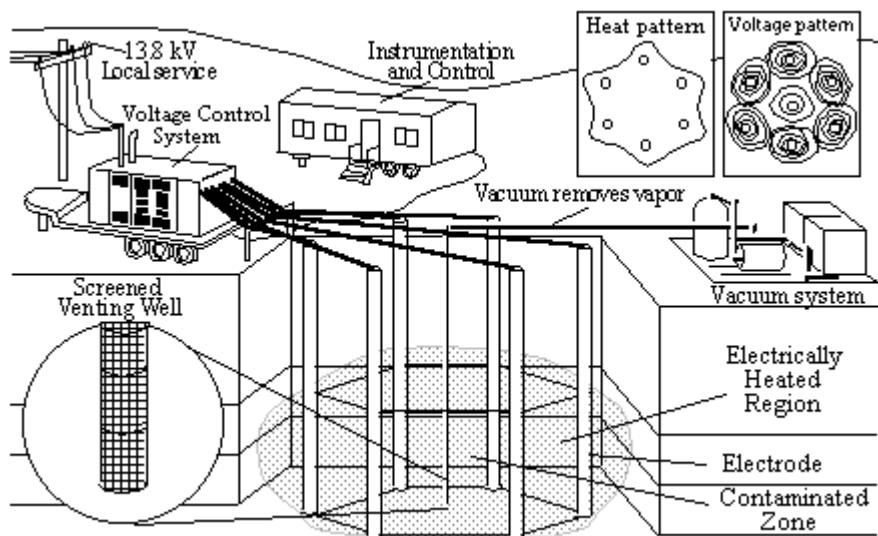
Figure 2-1. Illustration of Electrical Resistive Heating (ERH) application (from FRTR, 2002).

Electromagnetic heating refers to the absorption of electromagnetic energy by an object, which results in the excitation and rapid vibration of molecules. For example, electromagnetic energy in the microwave region (108 to 1012 Hz) causes water molecules to vibrate vigorously. Although microwave heating has been studied at the laboratory scale for treatment of PCB contaminated soils (Di et al., 2000) and other hazardous wastes (Wicks et al., 1999), it is unlikely that the technology will be implemented at the field scale for source zone remediation. However, electromagnetic energy in the radio frequency range (e.g., 0.5 to 45 MHz) can be absorbed by soils, which in turn generates heat in situ (U.S. Patent No. 4,670,634, Bridges et al., 1987). The technology was evaluated under the auspices of U.S. EPA Site program at several locations, most notably Kelly Air Force Base in San Antonia, TX (U.S. EPA, 1995a,b).

Table 2-1. Comparison of in situ thermal treatment technologies.

Category	Hot Water Injection	Steam Injection	Conductive Heating	Electrical Resistive Heating	Electromagnetic Heating
Heat Transfer	Convective	Convective	Conductive	Conductive	Radiative
Energy Source	Hot Water	Steam	Steel Well Casing	Electrical	Electromagnetic
Temp. Limit	100 °C	100-120 °C	500-800 °C	100-200 °C	100-300 °C
Fluid Injection	Liquid	Steam	None	None	None
Recovery Process	Mobilize	Mobilize/ Volatilize	Volatilize	Volatilize	Volatilize
Phase Recovered	Liquid	Liquid+Gas	Gas	Gas	Gas
Technology Example	Contained Recovery of Oily Waste (CROW)	Steam Enhanced Remediation (SER)	In-Situ Thermal Desorption (ISTD)	Six-Phase Heating™ (SPH)	Microwave; Radio Frequency
Advantages	No Phase Change, Low Cost	Large Heat Input, Energy Efficient	Low Volatility Contaminants	Low Cost, Low Perm. Layers	Uniform Heating, Low Perm.
Limitations	Preferential Flow, Low Efficiency	Pref. Flow, Cost, DNAPL, Safety	High Element Density (5/100 ft2)	Pref. Heating, Drying, Safety	Low Energy Transfer-Drying
Implementation DNAPL Source	UGI Columbia Brodhead Creek	Visalia, CA SRS, SC	Rocky Mtn Arsenal Alhambra Pole Yard	Fort Lewis, WA Paducah, KY	Kelly AFB, SRS, Kirkland AFB

2.3. CONTAMINANT PHASE DISTRIBUTION

When heat is introduced to the subsurface, physical-chemical properties of DNAPL can be altered substantially, which in turn impacts the contaminant distribution between the solid (soil), liquid (water) and gas (if present) phases. For example, the saturated vapor pressure (P_o) of TCE and PCE increases 30-50 times, from 0.098 to 2.92 atm and from 0.024 to 1.20 atm, respectively, as the temperature is increased from 25 to 100 °C. Similarly, the dimensionless Henry's Law constant (H) of TCE and PCE increases 15-20 times, from 0.42 to 6.3 and from 0.69 to 12.4, respectively, as the temperature is increased from 25 to 100 °C (Wilson and Clarke, 1994; Heron et al., 1998a). As a result, even with modest heating (50-70 °C), a sizable fraction of contaminant mass (> 50%) is likely to exist in the gas phase of low organic carbon content soils (≤ 0.1%) (Davis, 1997; Heron et al., 1998b). However, above temperatures of 40-50 °C experimental Henry's Law constant data and equilibrium sorption coefficients and sorption rate parameters for DNAPL contaminants are almost nonexistent, rendering estimates contaminant phase distributions during thermal treatment subject to considerable uncertainty. This lack of data at elevated temperature served as the primary justification for Task 1 of the project, which focused on the measurement of physical-chemical parameters and sorption behavior of PCE and TCE at tempatures of up to 100 °C, as well as the collection of soil and groundwater samples from field sites undergoing thermal remediation.

2.4. CHEMICAL REACTIVITY AND BYPRODUCT FORMATION

In the presence of dissolved oxygen, the complete conversion of dissolved-phase TCE to carbon dioxide (CO_2) and chloride ions (Cl^-) has been claimed to occur during thermal treatment, a process referred to as hydrous pyrolysis oxidation (HPO) (Leif et al., 1998, U.S. DOE, 2000).

The degradation of dissolved-phase PCE and TCE is thought to be initiated via an electron transfer reaction (Costentin et al., 2003), which results in the formation of dichloroacetylene (C_2Cl_2) as an intermediate compound regardless of redox conditions:

$$Cl_2C{=}CCl_2 + 2e^- \rightarrow ClC{\equiv}CCl + 2Cl^- \text{ (Substitution Reaction Elimination: } S_{RE}1) \qquad (2\text{-}1)$$

$$Cl_2C{=}CClH + HO^- \rightarrow ClC{\equiv}CCl + H_2O + Cl^- \text{ (Base Catalyzed Elimination - E1cb)} \qquad (2\text{-}2)$$
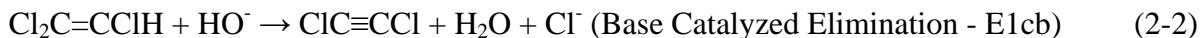
Dichloroacetylene (DCA) is a reactive compound that rapidly degrades. In heated environments with oxygen and water present (i.e., oxidizing conditions), DCA was proposed to react to form simple organic acids such as glycolate and formate, along with the carbon gases; carbon monoxide (CO) and CO_2 (Costanza et al., 2005):
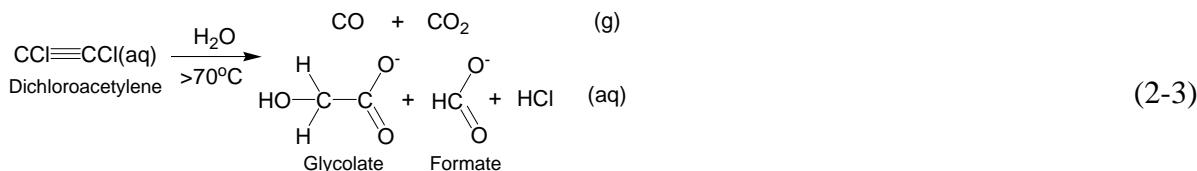
In reducing environments (e.g., when sulfide (S^{2-}) or ferrous iron (Fe^{2+}) are present), with a readily available source of hydrogen (H-donor), DCA would be expected react to form a

variety of compounds including TCE, the DCE isomers, ethylene, ethane, and C_4 compounds such as 1-butene (Figure 2-2). These compounds have been detected during the degradation of dissolved-phase PCE at room temperature (25 °C) in batch reactors that contained zero valent iron (Arnold and Roberts, 2000).
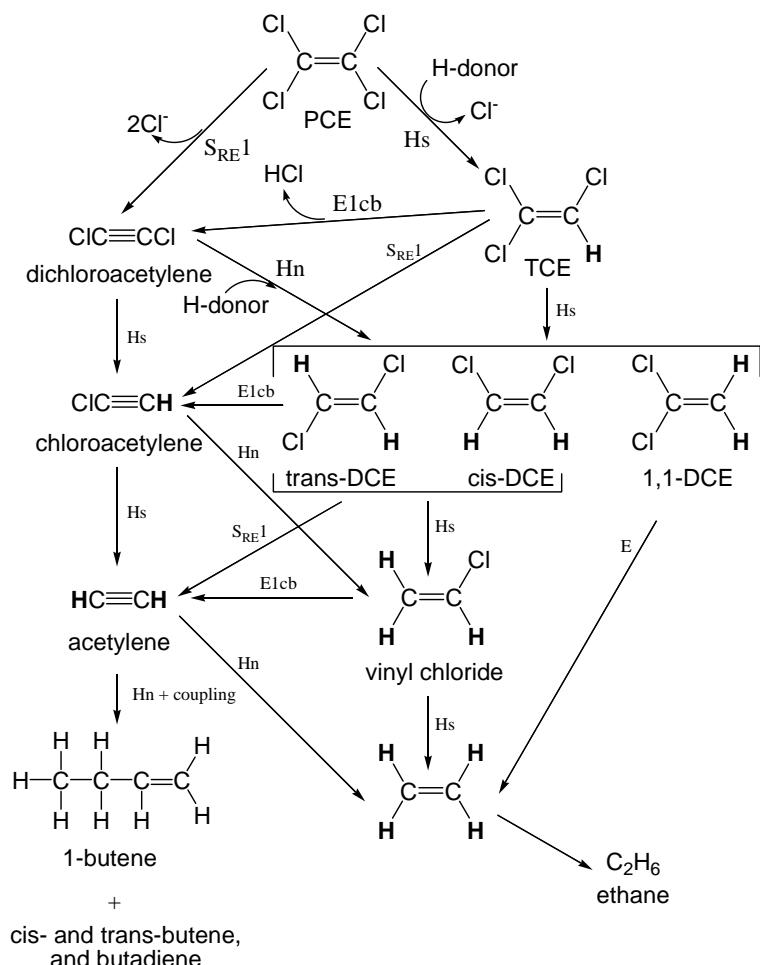
Figure 2-2. Summary of dissolved-phase chloroethene degradation reactions in reducing environments (adapted from Arnold and Roberts, 2000).

Potential chlorinated ethene degradation products that are expected to form in either oxidizing or reducing environments are listed in Table 2-2, and represent a minimum set of compounds that should be analyzed for when attempting to demonstrate the degradation of chloroethenes. Volatile organic degradation products, including TCE, DCE isomers, ethylene, ethane, acetylenes, and butanes, can be detected using gas chromatography (GC) methods. The detection of organic acids require ion chromatography (IC) and chloride can be determined using IC or colorimetric methods. Carbon monoxide and CO_2 gases can be determined using GC methods, but this requires a gas sample introduction loop and customized flame ionization detector (FID) for accurate determination. Analysis of the compounds listed in Table 2-2 was performed as part of the work reported herein.

10

Table 2-2. Summary of Potential PCE Degradation Products

Oxidation Products	Reduction Products
Cl^-	Cl^-
dichloroacetylene	TCE
CO	di- and chloroacetylene
CO_2	1,1-, cis-, and trans-DCE
glycolate/glyoxalate/acetate	vinyl chloride
formate	ethene
	ethane
	acetylene
	1-, cis-, and trans-butene
	butadiene
	n-butane

2.5. MICROBIAL REDUCTIVE DECHLORINATION

It is now recognized that even successful source zone treatment technologies will not completely remove DNAPL mass from source zones, and are likely to increase the mobility and distribution of the residual mass in the short term, as evidenced by increased aqueous phase concentrations. Thus, a key issue facing site managers is whether or not monitored natural attenuation (MNA) or enhanced passive remediation (EPR) can be relied upon to control plume development and migration following aggressive treatment of DNAPL source zones.

To date, the effects of thermal treatment on microbial community structure in general, and reductive dechlorination activity in particular, have only rarely been studied. Several of the early studies in this area focused on non-chlorinated organic contaminants, in particular polynuclear aromatic hydrocarbons (PAHs), that are subject to microbial degradation under aerobic conditions. Following steam treatment of a JP-5 jet fuel site at Naval Air Station Lemoore, microbial degradation resumed in zones that had reached temperatures of 80-100 °C for extended periods, but a shift in the microbial community structure was reported (Udell et al., 1994; Newmark and Aines, 1995). Laboratory studies demonstrated that mesophilic populations survived steam treatment, and the overall microbial metabolic activity was similar between controls and soils that were allowed to cool gradually (Richardson et al., 2002). This study also demonstrated that the microbial degradation of (PAHs) rebounded following steam treatment.

Although microbial characterization of subsurface environments following thermal treatment is limited, the limited monitoring data available suggest that aerobic microbial activity can rebound after field-scale steam injection. During steam or hot water injection, the subsurface will become aerobic, especially when air is co-injected to promote chemical oxidation or control downward migration of DNAPL existing free product. While the introduction of additional oxygen promotes aerobic degradation processes, oxic conditions will limit reductive dechlorination. Recent efforts demonstrated that microbial reductive dechlorination of PCE and TCE to nontoxic end products (i.e., ethene and inorganic chloride, Figure 2-3) can be a productive process at contaminated sites when anaerobic conditions are maintained (Ellis et al., 2000; Major et al., 2002; Ledvay et al., 2003; He et al., 2003a). At ambient temperaturates, recent studies indicate that (a) biostimulation via electron donor addition and (b) bioaugmentation with PCE-to-ethene-dechlorinating cultures are promising approaches that can promote chloroethene detoxification at sites where the rates of contaminant removal are insufficient (Ellis et al., 2000; Major et al., 2002; Ledvay et al., 2003).
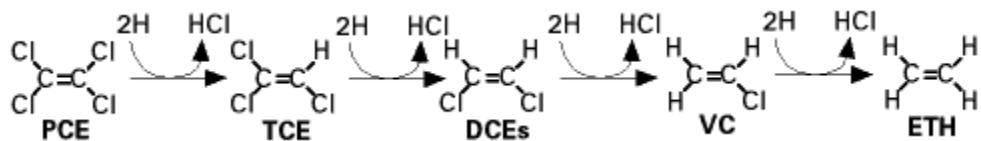
Figure 2-3. Reductive dechlorination of PCE to ethene and inorganic chloride (from Zinder and Gossett, 1995).

The key populations involved in the reductive dechlorination of chloroethenes belong to several bacterial groups including *Dehalococcoides*, *Dehalobacter*, *Desulfitobacterium*, *Desulfuromonas*, and *Geobacter*. Of particular interest is the *Dehalococcoides* cluster because members of this group are necessary to achieve reductive dechlorination of dichloroethene and vinyl chloride to ethene, and therefore, are critical for detoxification (Maymó-Gatell et al., 1997; Duhamel et al. 2002; He et al 2003a,b; Cupples et al., 2003). Microbial reductive dechlorination of chlorinated ethenes occurs most efficiently under anaerobic conditions, and thermal approaches compatible with subsequent reductive detoxification processes avoid the introduction of air or oxygen into subsurface environments. Field measurements of redox potential during electrical resistive heating suggest that some thermal technologies may be compatible with reductive dechlorination, which suggests that combining thermal with biological remedial approaches, either in parallel or in series, may provide more effective treatment options for DNAPL source zones (Beyke et al., 2000).

Furthermore, some studies have suggested that combining thermal treatment with biological remediation approaches may actually increase the efficiency of in situ reductive dechlorination. For example, it has been reported that organic carbon is released from the subsurface matrix during thermal treatment, increasing electron donor availability for dechlorinating organisms (Friis et al., 2005). Friis et al. (2005, 2006) suggest that biostimulation, the addition of electron donor to the subsurface, may not be required following thermal treatment due to the organic carbon released during thermal tretment. In previous microcosm studies performed by Friis et al. (2006), complete dechlorination of TCE to ethene

12

occurred in two of three previously heated microcosms both with and without electron donor addition. Conversely, in the same study, while complete dechlorination to ethene occurred in three of three unheated and biostimulated microcosms, complete dechlorination occurred in only one of three unheated microcosms that were not biostimulated. While these findings suggest that biostimulation may not be required to promote complete reductive dechlorination of PCE or TCE to ethene following thermal treatment, in their study, initial hydrogen concentrations in the majority of microcosms were at least three orders-of-magnitude greater than concentrations typically found in groundwater (Chapelle et al., 1996) due to hydrogen introduction during microcosm construction. Another potential beneficial effect of thermal treatment may be that hydrogen-consuming competitors to dechlorinating populations, such as methanogens, do not readily recover following exposure to elevated temperatures. For example, even after bioaugmentation with a methanogenic dechlorinating consortium, less methanogenesis was observed in previously heated microcosms than in those that were never heated, suggesting that competitors to dechlorinating populations are less successful following thermal treatment (Friis et al., 2006).

CHAPTER 3

MATERIALS AND METHODS

The following sections describe all of the materials and methods used during the completion of this project. The sections are organized by experiment type given that there were slight variations in procedures and analytical protocols between individual experiments.

3.1 HENRY'S LAW CONSTANT

Experiments undertaken to determine the Henry's Law constant of TCE with increasing temperature were performed in a 120 mL glass vessel manufactured by Ace Glass, Inc. (Vineland, NJ) and equipped with a threaded closure. The glass vessel was rated to hold pressures of up to 150 psig, operate at temperatures of up to 120 $^{\circ}$C, and had sufficient volume to accommodate soil. The vessel was sealed with a threaded Teflon® adaptor equipped with a Viton® O-ring (#5844-62, Ace Glass, Inc), which provided a sealed volume that was rated to hold a vacuum of 10^{-5} Torr. Gas and aqueous samples from the internal volume of the 120 mL vessel were collected through two sections of 1/8-inch outside diameter (OD) polyether ether ketone (PEEK) tubing. A photograph of the assembled equilibrium system is shown in Figure 3-1, illustrating the two sample collection tubes; one terminating in the aqueous phase and the other terminating in the gas phase.
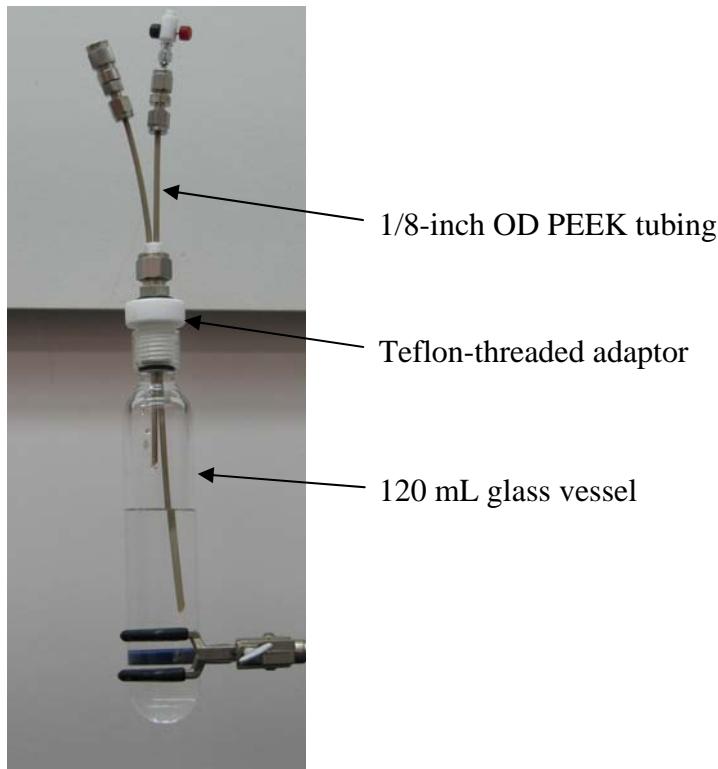
Figure 3-1. Photograph the gas-liquid equilibrium system.

The aqueous stock for the experiment was prepared using deionization (DI) water that was purified with a Milli-Q system (Gradient A10, Millipore Corp., Billerica, MA) to an electrical resistance of 18.2 MΩ/cm and total organic carbon content of less than 2 μg/L. The TCE-DI water stock was prepared by adding approximately 14 μL of 99.5% pure TCE (Sigma-Aldrich, Milwaukee, WI) to a 2 L volumetric flask containing the Milli-Q DI water and a Telfon®-coated magnetic stir bar. The 2 L flask was then sealed with a glass stopper and then placed on a magnetic stir plate where the contents were mixed at room temperature for at least 24 hours prior to use.

Five experimental trials were completed, and while there were slight differences between trials, the basic experiment consisted of filling the 120 mL vessel with between 50 an 90 mL of TCE containing DI water. Once filled, the 120 mL vessel was sealed and submerged in a 5 L glass vessel filled with DI water. The temperature of the DI water was controlled during the initial two trials using a stirring hotplate heater (Fisher Scientific, Pittsburg, PA) and then in final trials using a recirculation bath capable of controlling temperature to ± 0.01 °C (RTE 111, NESLAB Instruments, Inc., Portsmouth, NH). The 120 mL glass vessel, filled with TCE-containing water, was allowed to reach temperature equilibrium for 1 hour prior to sample collection. This time was found to yield stable gas-phase concentrations at incubation temperatures of 30, 40, and 50 °C.

Aqueous samples for the initial three experimental trials were collected using a 1 mL gas-tight (Hamilton Co., Reno, NV) syringe that was inserted through a polymer septa (Blue Septa, Alltech, Deerfield, IL), which, along with a 316 stainless steel union (Swagelok, Salon, OH), sealed the PEEK tube end. In subsequent experimental trials, aqueous samples were collected using a 1 mL gas-tight (Hamilton Co., Reno, NV) syringe connected to a Mininert valve (VICI Valco Instrument Co. Inc., Houston, TX), which sealed the PEEK tubing. It was found that the syringe had to be completely water filled to yield reproducible aqueous concentrations. This was accomplished by first removing a 1 mL aqueous sample from the 120 mL vessel to fill the syringe, the 1 mL sample was then discharged making sure to backfill the narrow bore of the Mininert valve stem. After filling the aqueous sample pathway, 3 samples were collected with a completely water-filled syringe. Aqueous samples were collected in 22 mL headspace vials filled with 10 mL of DI water and crimp-sealed with a 50-mm thick polytetrafluoroethene (PFTE)-lined silicone septa.

Gas samples were collected during the initial three experimental trials using a 1 mL volume gas-tight syringe with Teflon plunger that was equipped with a valve (SampleLock Syringe, Hamilton, Reno, NV) which was closed after the sample was collected to seal the gas sample within the syringe barrel while transferring the sample to the GC inlet. However, the use of a syringe to collect gas samples was problematic because the syringe needle was found to become blocked after penetrating the polymer septa (Blue Septa, Alltech, Deerfield, IL) that was used to seal the PEEK tube end, and thus, the exact volume of gas collected was uncertain.

3.1.1. Analytical Methods

Aqueous-phase concentrations of TCE were determined using an Agilent (Santa Clara, CA) 6890N Gas Chromatograph (GC) equipped with a Teledyne-Tekmar (Teledyne Technologies, Inc., Mason, OH) HT3 headspace autosampler and a 30 m long × 0.25 mm OD Agilent DB-5ms column connected to an Agilent inert Mass Select Detector (MSD). The headspace autosampler was programmed to hold each sample at 70 °C for a period of 15 min

prior to transferring the headspace gas to the GC injection port through silcosteel tubing heated to 110°C. Calibration standards were prepared by injecting small volumes of a 10,000 mg/L TCE-methanol stock solution into 22 mL headspace vials filled with 1 mL of DI water.
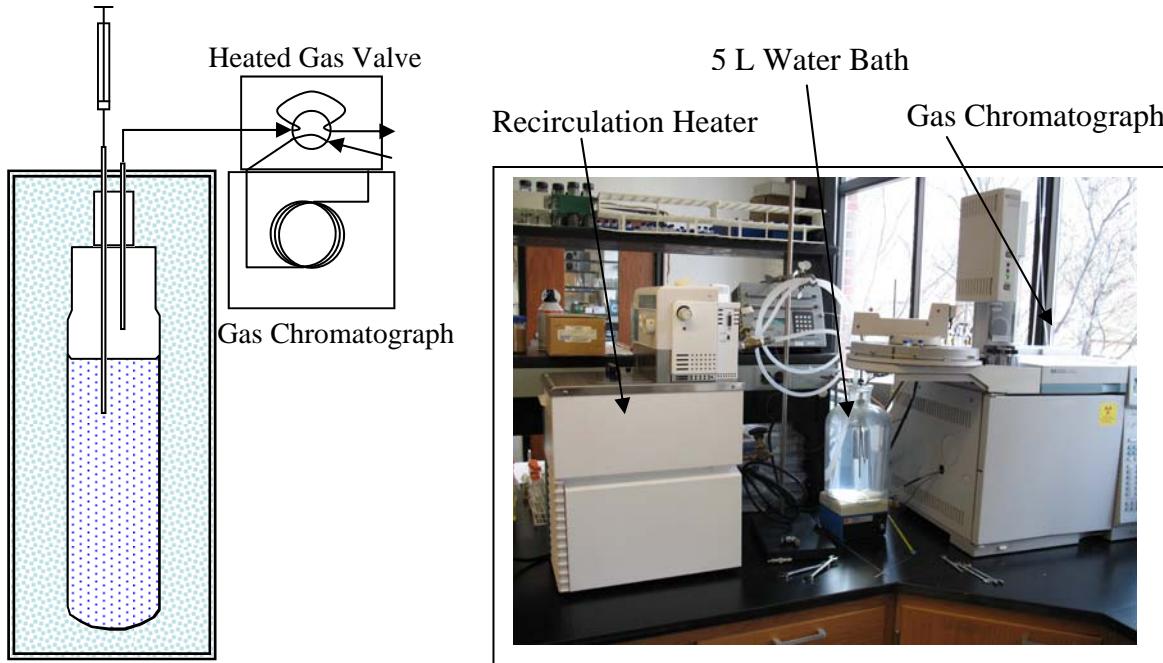
Figure 3-2. Diagram and picture of gas-liquid equilibrium system used during experimental Trials 4 and 5.

Gas samples for subsequent experimental trials were collected through stainless stell tubing connected directly to a GC sample valve heated to 120 °C and equipped with a 250 μL sample loop (Figure 3-2). The sample valve was located in a Hewlett-Packard (HP) Model 6890 GC equipped with a 30 m × 0.32 mm OD Agilent DB-5 column connected to a flame ionization detector (FID). Calibration of the GC-FID was performed by using methanol stock solutions prepared from a 5,000 mg/L TCE-methanol master stock. The gas-phase TCE concentration was calculated based on the mass of TCE introduced into the GC from 2 μL injections of the TCE-methanol calibration solutions (mass TCE/peak area) and the volume of gas collected from the 120 mL glass vessel (peak area/μL gas). The volume of gas sample collected was calculated using the ideal gas law, the sample loop volume of 250 μL and temperature of 120 °C, and equilibrium system temperature; $V_s = V_l*(T_s/T_l)$, where V_s is the volume of sample, V_l is the volume of liquid, T_s is the temperature of the sample, and T_l is the temperature of the liquid.

3.1.2. Equilibrium Partitioning in Closed System Experiments

An additional set of experiments was performed based on the Equilibrium Partitioning in Closed Systems (EPICS) method (Gossett, 1987), as modified for use with automated headspace samplers by Shimitori and Arnold (2002) and Chai and Zhu (1998). These techniques involve adding TCE containing aqueous solution to 22 mL headspace vials and analyzing the gas-phase TCE concentration using an automated headspace sampler. The Shimitori and Arnold (2002) method involved filling a headspace vial with between 1 and 16 mL of DI water, introducing 5 μL of a TCE-methanol stock, which was sealed without delay by affixing a Teflon lined crimp seal. A series of at least five vials was prepared with increasing volumes of DI water, but constant contaminant mass, yielding a series of vials with decreasing TCE concentration. The vials were then automatically incubated for 30 minutes and sampled by the HT3 autosampler.

The resulting data were analyzed by calculating the Henry's Law constants for pairs of vials using the modified EPICS equation as derived by Gossett (1987). Another automated headspace EPICS method was developed by Chai and Zhu (1998) that involved using vials filled with 0.5 and 10mL of water with the same TCE concentration in each vial. For the Chai and Zhu (1998) method, a stock TCE solution was prepared by adding small quantities (< 200 μL) of neat TCE into a 4 L glass bottle containing DI water and a magnetic Teflon®-coated stir bar, and fitted with a 20 mL bottle-top pipette (Lab Industries Repipet, Barnstead International, Dubuque, IA). After mixing the solution on a stirplate at 25°C for at least 12 hours, headspace vials were filled with the TCE stock solution and then sealed without delay by affixing a Teflon®-lined crimp seal. Three vials containing 0.5 mL and three vials containing 10 mL of the TCE stock solution were prepared and then analyzed using the HT3 automated headspace sampler.

3.2 AQUEOUS SOLUBILITY

In combination with the Henry's Law constant studies, a separate set of experiments was completed to measure the aqueous solubility of TCE with increasing temperature. The equilibrium system used to determine the Henry's Law constants (described in Section 3.1), which consisted of a 120 mL glass vessel equipped with a threaded closure, was used for determining TCE solubility. The experiments consisted of filling the 120 mL vessel with 20 mL of neat TCE followed by 20 mL of deionized water leaving 80 mL of gas phase. The vessel was sealed and then placed on a rocking platform mixer and allowed to mix for 8 days to achieve equilibrium at 25 °C. After equilibrating, the vessel was sealed with a threaded Teflon adaptor equipped with two sections of 1/8-inch OD stainless steel tubing allowing for the collection of aqueous and gas samples from within the vessel. A photograph of the assembled equilibrium system is shown in Figure 3-3, illustrating that one tube was terminated in the aqueous phase and the other in the gas phase. The assembled system was then submerged in a 5 L water bath connected to a recirculation heater capable of controlling the temperature to ± 0.01 °C (RTE 111, NESLAB Instruments, Inc., Portsmouth, NH). Two aqueous samples were collected from the vessel after maintaining a constant temperature for at least 1 hour. The isothermal temperatures used included 25, 40, 50, 60, 65, 70, 75, 80, and 85 °C to cover the range of temperatures expected to occur during in situ thermal treatment.

Gas

Water

TCE NAPL

1/8-inch OD stainless-steel tubing

Teflon-threaded adaptor

120 mL glass vessel
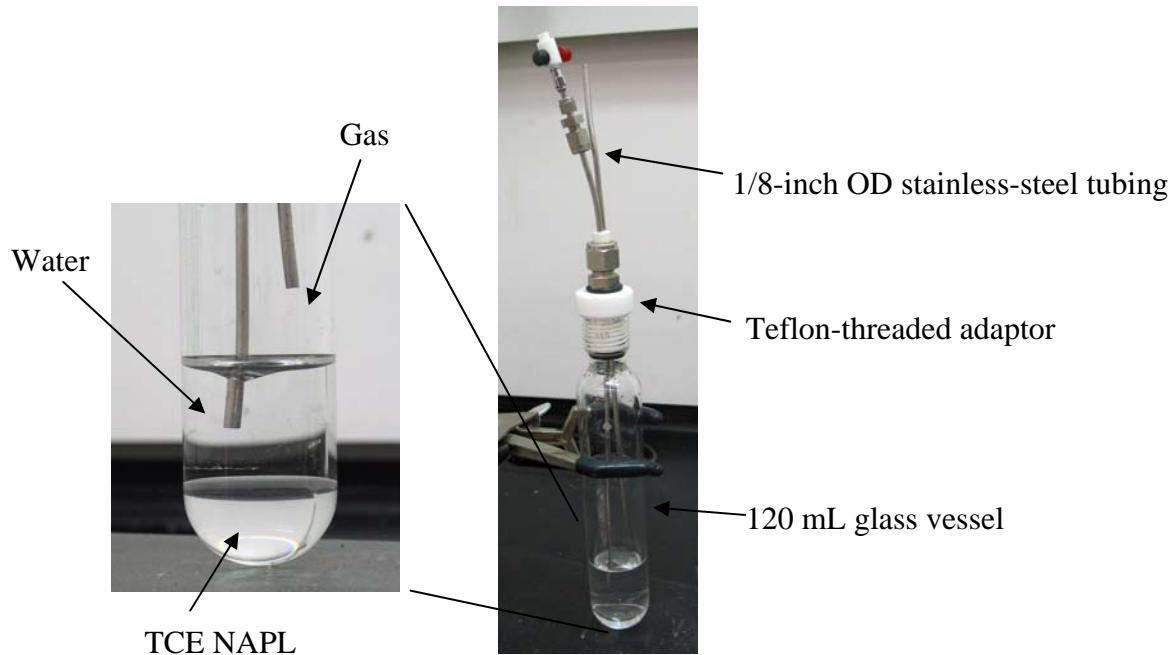
Figure 3-3. Photographs of the gas-liquid equilibrium system for measuring chlorinated ethene solubility.

3.2.1. Analytical Methods

Aqueous-phase concentrations of TCE were determined using an Agilent Model 6890N Gas Chromatograph (GC) equipped with a Teledyne-Tekmar HT3 headspace autosampler and a 30 m long $\times$ 0.25 mm OD Agilent DB-5 column connected to a Flame Ionization Detector (FID). The headspace autosampler was programmed to hold each sample at 70 ^{o}C for a period of 30 min prior to transferring the headspace gas to the GC injection port through silcosteel tubing heated to 150 ^{o}C. Calibration standards were prepared by injecting small volumes of a 20,000 mg/L TCE-methanol stock solution into 22 mL headspace vials filled with 1 mL of DI water.

3.3 COLLECTION OF FIELD SAMPLES

Soil samples from four field sites undergoing thermal treatment by electrical resisting heating were collected as part of this project (Table 3-1). Two of the sites, Camelot Cleaners Superfund Site and Great Lakes Naval Training Center Site 22, were former dry cleaning facilities. The East Gate Disposal Yard (EGDY) historically received spent solvents from the Fort Lewis Army Base maintenance operations, while the Pemaco Superfund Site was a former paint blending facility. Contaminated soils from Camelot Cleaners and Great Lakes Site 22 consisted of low permeability silt and clay while soils from the East Gate Disposal Yard were of greater permeability with a range of grain sizes from gravels down to clays. The soils from Pemaco were collected from above the groundwater aquifer in the water unsaturated or vadose zone and consisted of medium permeability sand to silt size grains.

18

Table 3-1. Field sites undergoing thermal treatment from which soil samples were collected.

Site Name	Location	Soil Type	Contaminant
Camelot Cleaners Superfund Site	West Fargo, ND	Clay	PCE
East Gate Disposal Yard, DNAPL Area #3	Fort Lewis, WA	Glacial Outwash (Gravel to Clay)	TCE
Great Lakes Naval Training Center, Site 22	Great Lakes, IL	Silty Clay	PCE
Pemaco Superfund Site	Maywood, CA	Silty Sand	TCE

3.3.1. Site #1: Camelot Cleaners Superfund Site, West Fargo, ND

Soil and groundwater samples were obtained from the Camelot Cleaners Superfund site located in West Fargo, ND by personnel from Current Environmental Solutions (CES), Kennewick, WA. Two soil cores (ca. 1 kg each) were collected 45 feet below ground surface (bgs) and from 56 feet bgs from a single borehole (E17) using a split-spoon sampler with acetate liner. The clay soil, classified as a Typic Eqiaquerts, was grey in color, very sticky and plastic, and adhered to glass when wet. The cores were transferred from acetate liners into autoclaved mason jars under anoxic conditions within an argon filled glove bag and 4 soil subsamples were collected from each core to determine the initial PCE content. Each soil subsample has a mass of approximately 15 grams and was placed in a 40 mL vial that contained 10 mL of methanol. The compounds present in methanol extracts of the 45 feet bgs soil core included PCE, with less than 1 mg/kg of toluene and xylene. The concentration of PCE was $1,082 \pm 924$ mg/kg based on 4 subsamples from the 45 foot bgs soil core.

Groundwater (1.4 L) was collected from a monitoring well (PMW-10) screened from 45 to 60 feet bgs, and located within 50 feet of E17 as no groundwater was present at E17. No contaminants were detected in the 56 feet bgs soil core or in the groundwater.

Thirty-two post-thermal treatment groundwater samples were collected by CES personnel from Process Monitoring Wells (PMW), which were used to determine ERH system performance during treatment of the Camelot site. The groundwater samples were collected by CES personnel on 28 November 2005 from nine PMWs at depths between 7 and 56 feet bgs. The groundwater samples represent post-thermal treatment conditions as they were collected after treating the site with ERH for approximately one year.

3.3.2. Site #2: East Gate Disposal Yard, Fort Lewis, WA

An initial round of soil samples was collected from the East Gate Disposal Yard, Fort Lewis, WA during the installation of flux-meter wells (FX Wells, Figure 3-4) by Army Corps of Engineers, Seattle District personnel between 14 June and 16 July 2005 (Table 3-2). These samples were transferred from rotosonic core bags into non-sterile mason jars and exposed to oxygen present in air. Therefore, there may have been an adverse impact on native bacteria that

are capable of reductively dechlorinating TCE, the primary contaminant at DNAPL Area #3. A second round of soil sampling was designed, in collaboration with Mike Truex from Pacific Northwest National Laboratories (PNNL), so that soil was transferred into sterilized mason jars within an argon atmosphere to minimize impact to dechlorinating bacteria. Soil samples were collected during the completion of borings RS0047b and RS0051b on 5 and 6 April 2006 (Figure 3-4). Samples from two locations were required because location RS0047b was amended with a whey solution to stimulate biotic reductive dechlorination (i.e., biostimulated), whereas location RS0051b represents un-amended soils. Samples from boring RS0047b were expected to contain greater numbers of reductive dechlorinating microbial species and cells than samples from boring RS0051b. The soil borings were completed using the rotosonic drilling technique which consists of vibrating a 10-inch OD steel casing into the ground, then retracting the steel casing, and extruding soil within the casing into plastic bags. The soil subsamples were collected after transferring a plastic bag of soil, which represented a 2-foot interval of boring, into a disposable glove bag filled with argon. The plastic bag was sliced open and soil from the bag was transferred to a pre-sterilized polypropylene quart jar. After soil transfer, the jar was filled with groundwater collected from monitoring well FX3-02 to minimize exposure to oxygen during shipment from Fort Lewis, WA to Atlanta, GA. The groundwater was passed through sterile filters (1.0 and 0.2 μm pore size filters) before collection into pre-sterilized 2 L polypropylene screw-top bottles. Soil was collected from four depth intervals from RS0047b and RS0051b (Table 3-2), for depth intervals corresponding to the known TCE source zone (18 to 20 feet bgs) and are from depths where no previous samples had been collected (44 to 46 feet bgs).

Table 3-2. Soil and water samples from the East Gate Disposal Yard (EGDY), Fort Lewis, WA.

Sample Collected Event	Soil Sample Locations	Depth (ft bgs)	Water Sample Locations
Initial soil and water samples collected by Army Corps personnel 14 June and 16 July 2005.	FX3-03: 13-13.5	13	FX3-03 (1 L)
	FX3-03: 15.5-16.5	15.5	
	FX3-03: 34-36	34	
	FX3-02: 29.5-30.5	29.5	FX3-02 (1 L)
	FX3-02: 38-39	38	
	FX3-18: 24	24	
	FX3-01: 12-12.5	12	FX3-01 (2 L)
	FX3-01: 24.5-25.5	24.5	
	FX3-17: 38	38	
	FX3-04: 29	29	FX3-04 (1 L)
	FX3-19: 23	23	
Second round of soil and water samples collected by ER-1419 personnel 5 an 6 April 2006.	RS0047b	18-19	FX3-02 (Passed through 0.2 um pore size filter – "Field Sterilized")
		28-30	
		34-36	
		44-46	
	RS0051b	18-20	
		28-30	
		34-36	
		44-46	

Figure 3-4. Map of East Gate Disposal Yard DNAPL Area #3 showing relative location of soil and groundwater samples. The initial soil samples were collected during the installation of flux-meter wells (FX Wells) and the second round of samples were collected during installation of RS0047b and RS0051b. The FX wells are approximately 20 feet apart.

3.3.3. Site #3: Great Lakes Naval Training Center, Great Lakes, IL

Soil samples from Site 22, a former dry cleaning facility, located at the Naval Training Center Great Lakes, Great Lakes, IL were collection prior to and after electrical resistive heating (pre- and post-ERH) thermal treatment. The samples were collected by TetraTech NUS, Inc. personnel between 25 and 27 April 2006 from location F3 at depths between 4 and 20 feet bgs and location C2 between 9 and 11.5 feet bgs (Table 3-3 and Figure 3-5). Soil samples from location F3 represented the portion of the site where the greatest concentrations of PCE were detected while soil from location C3 had relatively lower concentrations of PCE. The soil samples were collected during the installation of the ERH electrodes using hollow stem auger methods and soil samples were retrieved using steel tubes. The soil, which consists of grey silt to clay size grains, was extruded from the steel tubes into plastic zip-lock freezer bags which were sealed, then placed in ice chests, and shipped overnight from Great Lakes, IL to Atlanta, GA. After collecting the soil samples, groundwater water was collected from wells MW06S and MW10S located near soil sample location F3 and from MW05S located near soil sample location C2.

Table 3-3. Soil and water samples collected from Site 22, Naval Training Center, Great Lakes, IL.

Sample Collected Event	Soil Sample Locations	Depth (ft bgs)	Water Sample Locations
Pre-thermal treatment by TetraTech NUS personnel 25 and 27 April 2006.	F3	4-6 6-8 8-10 10-12 16-18 18-20	MW06S/MW10S
	C2	9-11.5	MW05S
Post-thermal treatment by TetraTech NUS personnel 14 September 2006.	MW10D	18-19 28-30 34-36 44-46	MW10D
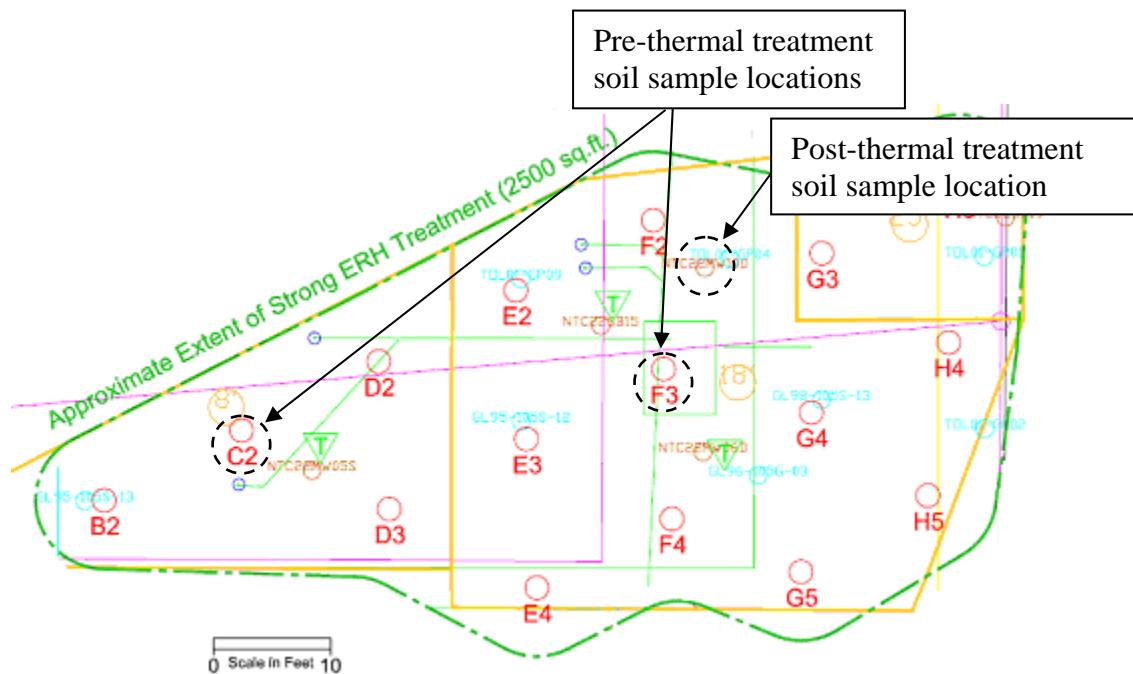
Figure 3-5. Map of Great Lakes Naval Training Center, Site 22 showing the location of pre-thermal treatment soil samples (C2 and F3) and the post-thermal treatment sample (MW10D).

Post-ERH thermal treatment soil samples were received from the Naval Training Center Great Lakes, IL on 14 September 2006. Soil samples were collected by TetraTech NUS, Inc. personnel on 12 September 2006 during the installation of monitoring well MW10D, at depths between 4 and 12 feet bgs. These samples were from a location on Site 22 that had the greatest

concentration of PCE prior to thermal treatment. Groundwater samples from well MW10D were collected once the site had cooled to ambient temperatures (25 ^{o}C) in December of 2006.

3.3.4. Site #4: Pemaco Superfund Site, Maywood, CA

Soil samples were collected during the installation of an ERH system designed to heat a layer of silty sand located between 55 and 60 feet bgs. The silty sand layer is contaminated with TCE and is located approximately 60 feet above the groundwater aquifer. The samples were collected by TN & Associates, Inc. personnel on 13, 14, and 19 November 2006 during the installation of pre-thermal treatment borings using hollow stem auger methods. Soil samples were retrieved in acetate sleeves that were cut into one foot sections, the open ends of the sleeves were sealed with parafilm, and then capped with plastic end pieces. The samples were shipped overnight in ice chests from Maywood, CA to Atlanta, GA the same day they were collected. Soil samples were collected from two boreholes within the ERH treatment area (TMP-9 and -17) and from one from a borehole (TMP-27) located outside the treatment area (Table 3-4, Figure 3-6).

Table 3-4. Soil Samples from Pemaco Superfund Site, Maywood, CA

Sample Collected Event	Soil Sample Locations	Depth (ft bgs)	Water Sample Locations
Pre-thermal treatment by T N & Assoc. personnel 13, 14, and 19 November 2006.	TMP-17	45-50	No water water unsaturated soils (vadose zone)
		55-60	
	TMP-9	46-49	
		55-60	
	TMP-27	41-48	
		55-60	
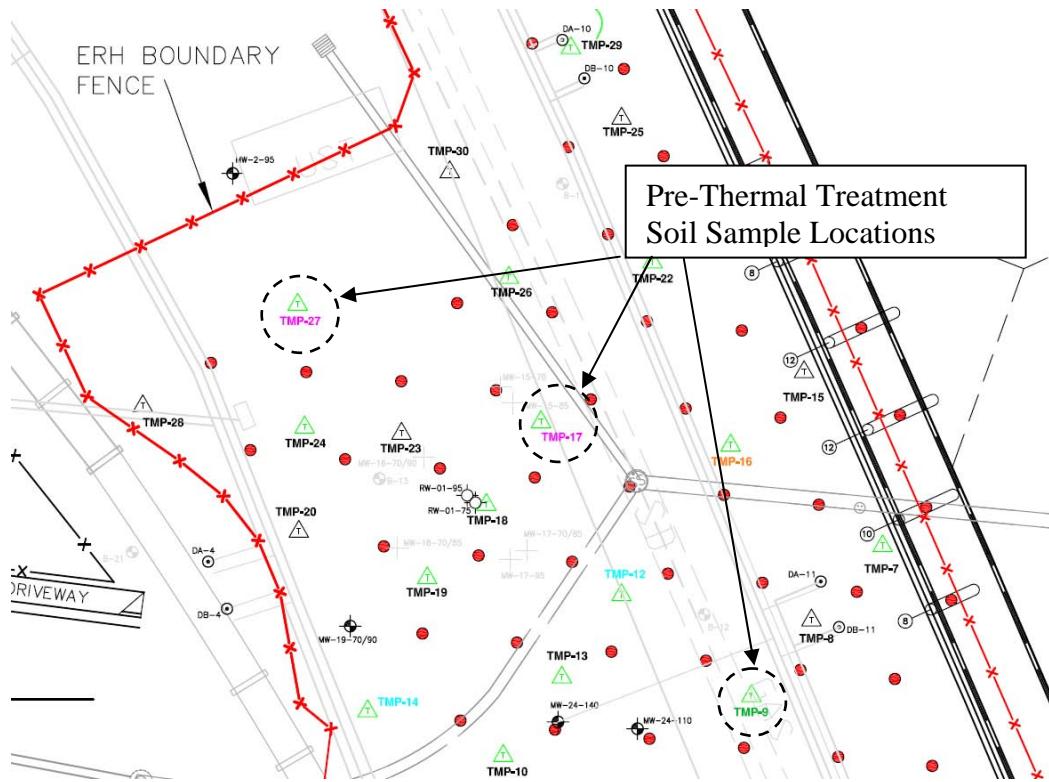
Figure 3-6. Map of Pemaco Superfund site showing the location of pre-thermal treatment soil samples (TMP-9, -17, and -27).

3.4 AMPULE BATCH EXPERIMENTS

Batch experiments were performed to determine the rate and extent of chloroethene degradation as a function of temperature and incubation time. Batch experiments were conducted in clear, 25 mL borosilicate glass ampules (Kimble-Kontes, Vineland, NJ) that were autoclaved prior to use. Ampule batch experiments consisted of filling and sealing autoclaved ampules during a one day period. The ampule sealing process involved using a propane-oxygen torch (BernzOMatic, Medina, NY) that had a maximum flame temperature of ca. 2,500°C. The flame sealing process consisted of heating the ampule neck using the outer (cooler) portion of the torch flame to vaporize any water droplets (ca. 10 s), followed by melting of the glass with the inner (hotter) portion of the torch flame (ca. 5 s). After flame sealing, ampules were placed in temperature controlled containers to undergo incubation. Following incubation over periods ranging from 5 to 288 days, ampules were destructively sampled to determine the chemical composition of each ampule phase (i.e., gas, aqueous, and solid). The destructive sampling process involved inverting each ampule (i.e., flamed sealed tip downward) followed by breaking the neck by hand. The opened ampule was then placed in a sampling device fitted with a 30 cm long needle. A stream of N_2 gas passed through the sampling device at a rate of 500 mL/min to minimize the introduction of air during sample collection. Aqueous samples were immediately collected from the ampule neck to determine the concentration of dissolved oxygen for the DI

water containing ampules, and for the concentration of ferrous and ferric iron, and sulfide in the soil containing ampules. A 2.5 to 5 mL gas sample was then collected from the main ampule body using a gas-tight syringe (Becton Dickinson, Franklin Lakes, NJ) for immediate injection into gas chromatographs. Following gas sampling, a 1 mL aqueous sample was collected from the inverted ampule by gas tight syringe and transferred to headspace vial for GC analysis using a headspace sample introduction system. Ampule contents during these experiments ranged from analytical grade water to samples from each of the four field sites (Table 3-5).

Table 3-5. Summary of ampule batches prepared for Project ER-1419.

Batch Contents	Total No. of Ampules	Incubation Temperatures	Max. Time (days)
DI water only with PCE	160	25, 55, 75, and 95°C	67
Ottawa sand and goethite with TCE	77	25 and 120°C	288
Iron(+II), manganese(+II) and sulfide(−II) Ion ampules with PCE and TCE	110	70°C	215
Camelot Cleaners Superfund Site samples	146	25, 55, 75, and 95°C	75
East Gate Disposal Yard, DNAPL Area #3 samples	110	25, 50, and 95°C	95.5
Great Lakes Naval Training Center, Site 22 samples	88	25, 50, 70, and 95°C	185
Pemaco Superfund Site samples	80	25, 50, 70, and 95°C	188
Totals	771		1113.5

These experiments involved the preparation of over 770 individual ampules and 3 years of cumulative incubation time. The following sections describe the materials and methods used for each of the ampule batch experiments to account for slight variations between each experiment.

3.4.1. Reactivity of PCE in Water Alone and Camelot Soil

An initial batch of 80 ampules was prepared with DI water that contained 7.1 ± 0.9 mg/L of PCE, where the ampule oxygen content was minimized (anoxic). And a second batch of 80 ampules was prepared with DI water and contained 7.0 ± 0.6 mg/L of PCE with the oxygen content near ambient levels (oxic). The water used to prepare the solutions was obtained from a Nanopure® analytical deionization system (model D4741, Barnstead International, Dubuque, IA). The DI water was used after dispensing through a 0.2 μm pore size filter without the addition of pH buffers. Low dissolved-oxygen content water, referred to as anoxic water, was prepared by sparging freshly-dispensed DI water with purified argon gas (Airgas-South, Inc., Marietta, GA) for at least 1 h. The dissolved oxygen content of anoxic water was between 0.2 and 0.3 mg/L as indicated by the Rhodazine D method (CHEMetrics, Calverton, VA). Oxygen-saturated water, referred to as oxic water, was prepared by sparging DI water with ultra zero

grade air (UZA, Airgas-South, Inc., Marietta, GA) for at least 1 h to yield dissolved oxygen concentrations between 8 and 10 mg/L as indicated by the Indigo Carmine method (CHEMetrics, Calverton, VA).

Stock PCE solutions were prepared in 2 L flasks that were filled with either anoxic or oxic DI water. Neat PCE (13 µL) was added to the 2 L flasks to create a 10 mg/L solution without the use of a co-solvent. The 2 L flasks were then sealed and covered with a dark plastic bag to minimize light exposure. The contents of the flasks were mixed with a Teflon-coated magnetic stir bar for a period of at least 36 h at room temperature (25 ± 1 °C).

Prior to use, the ampules were autoclaved for 25 min at 121 °C and then rinsed with DI water. The ampules were placed in a glass desiccator that was evacuated under a vacuum of 750 mm Hg for a period of 1 h and flushed with either argon gas or ultra zero grade air (UZA) to achieve anoxic or oxic conditions, respectively. The ampules were filled with approximately 20 mL of the 10 mg/L PCE stock solution, which was dispensed under positive pressure from the appropriate (i.e., anoxic or oxic) 2 L flask. Immediately prior to flame sealing, 1 mL water samples were collected from 4 randomly selected DI water filled ampules to determine the initial aqueous phase PCE concentration.

For ampules containing solids, Camelot soil was loaded prior to the addition of the aqueous phase resulting in 8.7 ± 1.4 g of soil per ampule. Prior to loading into ampules, the soil was air-dried and homogenized to create a more uniform initial PCE content, and to prevent wet soil from adhering to the ampule neck during loading. To dry the soil, the cores were placed in a sterilized glass tray and left in a laminar flow hood for 12 hours at room temperature (25 ± 1 °C). The soil was then homogenized using a mortar and pestle to break up clumps and then thoroughly mixed in the sterilized glass tray. The average concentration of PCE in the soil after being homogenized was 14.7 ± 7.0 mg/kg based on the analysis of methanol extracts of 12 randomly collected soil samples.

Groundwater samples were collected in four, 1-pint glass canning jars sealed with standard canning lids; no air bubbles were evident in the water samples and the samples were not filtered. The groundwater was collected from a monitoring well located at the Camelot site because no groundwater was present at location E17 where the soil samples were collected. The groundwater was stored at 4 °C until used during preparation of the ampules. Prior to filling the ampules, the groundwater was transferred to a 2 L glass flask that had been autoclaved and then rinsed with DI water. Approximately 600 mL of DI water was added to the groundwater to bring the total volume to 1.6 L, enough so that each ampule would receive 15 mL of aqueous solution. A Teflon®-coated stir bar that had been rinsed with methanol was added to the flask, and the flask was placed on a magnetic stir plate to mix the groundwater solution while transferring 15 mL aliquots of water into each ampule.

Ampules were incubated at 25, 55, 75, and 95 °C in custom-made enclosures that consisted of ice chests fitted with 1/4-inch inside diameter (ID) copper tubing. The copper tubing was connected to constant temperature recirculation bath heaters (Neslab RTE-211 and RTE-111) filled with food grade mineral oil (STE Oil Co., San Marcos, TX). Enclosure temperatures were recorded every hour using a K-type thermocouple connected to a data logger (Campbell Scientific, Inc., Logan, UT). The average enclosure temperatures were 53.7 ± 0.6, 77.1 ± 0.5, and 96.7 ± 0.6 °C. Ampules were also stored at room temperature (25 ± 1 °C) in an enclosed container. Ampules were removed from their controlled temperature environments after 5, 17,

37, and 75 days of incubation time and cooled to 25 °C to facilitate destructive sample collection.

3.4.2. Reactivity of TCE with Ottawa Sand and Goethite

An experiment was completed with TCE and Ottawa sand with goethite (1% wt.) incubated in ampules at 120 °C for a period of up to 288 days. This experiment was performed to confirm that iron bearing minerals can increase the rate of thermal induced TCE degradation as observed in previous experiments (Costanza et al., 2005).

The solids composition consisted of a mixture of 20-30 mesh Ottawa sand and 1% goethite. The solids were prepared by soaking approximately 2,000 g of sand from Ottawa, IL (ASTM 20-30 Sand, U.S. Silica Co., Berkeley Springs, WV) in 1 N nitric acid for 30 minutes to remove organic carbon and metals. The 1 N nitric acid soaking process was repeated, and then the sand was rinsed in DI water. The DI rinse consisted of placing small volumes of sand into the top of a 20-30-100 mesh ASTM sieve stack and running DI water over the sand. The sand was then placed back into a drying tray and DI water was added to cover the sand. The pH of the DI-Nanopure water covering the sand was measured with a pH probe (Accumet Model 50, Fisher Scientific, Fair Lawn, NJ) and the water rinse was repeated until the pH of the standing DI-Nanopure water was neutral (pH = 7). The sand was then placed into a drying oven and heated to 130 °C for 3 h to remove excess moisture and then baked at 200 °C for 2 hours to oxidize all transition metals. The oven temperature was lowered to 100 °C and the sand was allowed to cool for 3 h.

Approximately 500 g of the acid-washed sand was placed into a second glass drying tray to which 5 g of goethite powder was added to create a uniform 1% (wt) mixture. Research grade goethite chips, approximately 1 gram each, were obtained from Ward's Natural Science (Rochester, NY), and were reported to have been collected from Grants County, New Mexico. The goethite chips were ground into a fine power (silt to clay size particles) using a mortar and pestle prior to mixing with the sand. The drying trays were then autoclaved with steam at 17 psi (121 °C) for 25 minutes and the water from the autoclave process was allowed to vent from the trays for a period of approximately 30 minutes. Ampules that had been autoclaved and cooled in a desiccator where loaded with approximately 5.6 g of Ottawa sand and goethite (1% wt.) and temporarily sealed with aluminum foil.

The stock TCE solution was prepared in a 2 L flask that was filled with anoxic DI water. Neat TCE (137 µL) was added to the 2 L flask to create a 100 mg/L solution without the use of a co-solvent. The 2 L flask was then sealed and covered with a dark plastic bag to minimize light exposure. The contents of the flask were mixed with a Teflon®-coated magnetic stir bar for a period of at least 36 h at room temperature (25 °C).

Prior to use, the ampules were autoclaved for 25 min at 121 °C and then rinsed with DI water. The ampules were placed in a glass desiccator that was evacuated under a vacuum of 750 mm Hg for a period of 1 h and flushed with nitrogen to achieve anoxic conditions. The ampules were filled with approximately 14 mL of the 100 mg/L TCE stock solution, which was dispensed under positive pressure from the 2 L flask. Immediately prior to flame sealing, 1 mL water samples were collected from 12 randomly selected ampules to determine the initial aqueous phase TCE concentration of 83.5 ± 5.1 mg/L.

Ampules were incubated at 120 °C in a custom-made enclosure that consisted in a custom-made enclosure that consisted of an insulated box fitted with 1/4-inch ID steel tubing.

The steel tubing was connected to a constant-temperature recirculation bath heater (Neslab RTE-211) filled with silicone oil. The enclosure temperature was recorded every day using a K-type thermocouple connected to a data logger (Campbell Scientific, Inc., Logan, UT) and the average enclosure temperatures was 120 ± 5 °C. Ampules were also stored at room temperature (25 ± 1 °C) in an enclosed container. Ampules were removed from their controlled temperature environments after 2.2, 5.8, 9.8, 21.8, 46.8, 112.8, 141.4, and 287.8 days of incubation time and cooled to 25 °C to facilitate destructive sample collection.

3.4.3. Effect of Ionic Species on PCE and TCE Reactivity

An experiment was conducted to determine if common groundwater ions could increase the rate of PCE and TCE degradation during thermal treatment. The experiment involved preparing ampules with PCE and TCE containing solutions, amending the solutions with ferrous iron (Fe^{2+}), manganese (Mn^{2+}), and sulfide (S^{2-}), and incubating the sealed ampules at 70 °C for a period of 13 days.

De-ionized water used to prepare the solutions was obtained from a Milli-Q purification system (Gradient A10, Millipore Corp., Billerica, MA) after treatment to an electrical resistance of 18.3 MΩ/cm and a total organic carbon content of less than 2 µg/L. The DI water was used after dispensing through a 0.22 µm pore size filter without the addition of pH buffers. The Milli-Q DI water was sparged with nitrogen gas for 2 h until the dissolved oxygen concentration was less than 0.4 mg/L based on the Rhodazine D method (CHEMetrics, Inc. Calverton, VA).

The stock PCE and TCE solutions were prepared in separate 2 L flasks that were each filled with anoxic DI water. Neat PCE (13 µL) was added to one of the 2 L flasks to create a 10 mg/L solution without the use of a co-solvent and TCE (137 µL) was added to the second 2 L flask to create a 100 mg/L solution. Each 2 L flask was then sealed and covered with a dark plastic bag to minimize light exposure. The contents of the flasks were mixed with a Teflon®-coated magnetic stir bar for a period of at least 36 h at room temperature (25 °C).

Ion stock solutions were prepared in 100 mL volumetric flasks using nitrogen sparged DI water. Iron (II) sulfate heptahydrate (>99%, certified ACS) and manganese (II) sulfate monohydrate (>98%, certified ACS) were obtained from Fisher Scientific (Fair Lawn, NJ), while sodium sulfide nonahydrate (>98%, certified ACS) was obtained from Acros Organics (Geel, Belgium). The solutions were used immediately after preparation to minimize oxidation of the reduced transition metals.

Prior to use, 40 ampules were autoclaved for 20 min at 121 °C and then rinsed with DI water. The ampules were placed in a glass desiccator that was evacuated under a vacuum of 750 mm Hg for a period of 1 h and flushed with argon gas to achieve anoxic conditions. Twenty ampules were then filled with approximately 20 mL of the 10 mg/L PCE stock solution and the remaining 20 ampules were filled with 20 mL of the 100 mg/L TCE stock solution. To minimize the oxygen content of the solutions, argon was used to displace the solution from each 2 L flask into the ampules. After filling the ampules with PCE stock solution, 5 of the 20 ampules received 0.5 mL of anoxic DI water to serve as controls while 5 ampules received 0.5 mL of the 68 mM ferrous iron solution, 5 received 0.5 mL of the 72.2 mM manganese solution, and 5 received 0.5 mL of the 14.1 mM sulfide solution. The ampules were then flame sealed and placed in a water bath operated at 70 ± 3 °C. The ampules filled with TCE stock were then

prepared identically to those with PCE stock with the exception that 1 mL of DI water and each ion stock solution was used for the amendment.

After a period of 13 days, the ampules containing ferrous iron changed from a clear solution to one with an orange color potentially indicating the formation of iron oxyhydroxides and that a redox reaction had occurred with iron transitioning from the +II (ferrous iron) to the +III (ferric iron) oxidation state. The ampules were removed from the water bath at this point and cooled to 25 °C for destructive sampling.

A second related experiment was completed using post thermal-treatment groundwater obtained from the Camelot Cleaners site, which contained ferrous iron and sulfide. The experiment consisted of filling 70 autoclaved ampules with approximately 15 mL of groundwater collected from PMW 1, 2, 5, and 10 (see Section 3.3.1), and then amending the groundwater with 50 µL of a methanol stock solution that contained 3,382 mg/L PCE and 6,350 mg/L TCE. Ampules were incubated in a water bath maintained at 70 °C and in an enclosure at 25 °C for periods of 33, 128, 214, and 215 days.

3.4.4. Reactivity of TCE with Fort Lewis Soil

Soil and groundwater samples were obtained from the East Gate Disposal Yard as described in Section 3.3.2. The soil consisted of well-graded gravel in a matrix of sand, silt, and clay that was deposited as glacial till and outwash during the last Pleistocene glaciation period (Dinicola, 2005). The soil had low total carbon content of $0.069 \pm 0.005\%$ by dry combustion method (LECO CNS-2000), and low specific surface area of 8.8 ± 0.7 m^2/g by nitrogen gas adsorption (Micrometrics ASAP 2020), which decreased to 3.8 ± 0.6 m^2/g after treatment with 1M acetic acid at pH 5 to remove carbonates (Leoppert and Suarez, 1996; analyses performed by The Laboratory for Environmental Analysis, University of Georgia, Athens, GA). Soil samples were collected while installing boring RS0047b using the rotosonic drilling technique, which involves vibrating a 10-inch OD steel casing into the ground, retracting the steel casing, and extruding soil from the casing into plastic bags. Soil was collected from 28 to 30 feet bgs within the TCE-contaminated source zone.   Soil sub-samples were collected in pre-sterilized polypropylene quart jars after transferring the 2-foot core into a disposable glove-bag filled with argon gas to minimize exposure of the soil to oxygen.   Each quart jar was filled with groundwater collected from monitoring well FX3-02 to minimize exposure to oxygen during shipment. Groundwater was collected into pre-sterilized 2 L polypropylene screw-top bottles after passing through sterile 1.0 and 0.2 µm pore-size membrane filters (Whatman, Clifton, NJ). The concentrations of TCE and *cis*-DCE in the quart jars containing soil from boring RS0047b and groundwater from FX3-02 were 0.65 mg/L and 0.13 mg/L, respectively.

Batch experiments were conducted in clear, 25 mL borosilicate glass ampules (Kimble-Kontes, Vineland, NJ). Prior to use, the ampules were autoclaved for 25 min at 121 °C, placed in a glass desiccator which was evacuated at 750 mm Hg for 1 h and then filled with argon gas. Within an argon-filled glove bag, 38 ampules were each loaded with ca. 15 g of soil from boring RS0047b followed by 10 mL of groundwater. To facilitate loading of the ampules, soil particles greater than 4 mm in diameter were removed by passing the soil through a sterilized sieve (ASTM No. 5 sieve) within an argon-filled glove bag. After adding soil and water, each ampule was spiked with 10 µL of 20,000 mg/L TCE-methanol stock or 1.5 µmol of TCE per ampule, temporarily sealed with aluminum foil, and then permanently flame-sealed by melting the top 1 cm of the ampule neck with a propane-oxygen torch. While TCE was initially present in the

quart jar with soil collected from boring RS0047b, the process of sieving and transferring aliquots of the sandy soil was anticipated to reduce ampule concentrations to near detection levels of 0.1 mg/L thereby necessitating TCE amendment. An additional 21 solids-free ampules were prepared with 20 mL of groundwater and 10 μL of TCE-methanol stock amendment. Thirty-six TCE-free vials were prepared, without TCE or methanol amendment, using 22 mL glass headspace vials filled with ca. 12 g of EDGY soil and 10 mL of groundwater to determine native chloride levels. The 22 mL headspace vials were sealed with PTFE-faced stoppers (Kimble/Kontes, Vineland, NJ) and secured with crimped aluminum seals.

Ampules were sequentially numbered during preparation and then assigned an incubation temperature based on a random numbering system to minimize bias introduced during sequential preparation. Ampules were incubated at 25 ± 1 °C in an enclosed container, 50 ± 3 °C in a water bath (Thermo Electron Corp., Marietta, OH), or 95 ± 3 °C in a custom-made enclosure that consisted of an insulated box fitted with 1/4-inch ID steel tubing. The steel tubing was connected to a constant-temperature recirculation bath heater (Neslab RTE-211) filled with silicone oil. Eight ampules were placed in the 25 °C container (2 ampules × 3 incubation times and 1 ampule × 2 incubation times), while 15 ampules each were incubated in the 50 °C water bath and 95 °C insulated box (3 ampules × 5 incubation times). Ampules were removed from their controlled temperature environments after 10, 23.6, 42, 66.6, and 95.5 days of incubation time and cooled to 25 °C to facilitate destructive sample collection. The mass of TCE associated with the solid phase in each ampule was determined using a single solvent extraction step that consisted of adding 15 mL of methanol to each ampule after draining the free water, followed by transfer of the soil-methanol slurry to a screw-top vial that was maintained at 25 ± 3 °C for at least 24 h prior to analyzing the methanol for TCE content.

3.4.5. Reactivity of PCE with Great Lakes Soil

Soil and groundwater samples were obtained from the Naval Training Center Great Lakes as described in Section 3.3.3. The Great Lakes soil consists of heavy clay, which was not amenable to transfer through the narrow ampule neck opening. Instead of drying the soil, as was done with the Camelot soil (see Section 3.4.1), a slurry was prepared by adding soil from boring F3 collected between 8 and 10 feet bgs and groundwater from monitoring well MW6S in equal volumes to a mason jar and mechanically breaking the clay into smaller fragments with a spatula followed by vortex mixing. The clay slurry was prepared over a 1-month period and yielded a uniform solution that contained ca. 1 g soil per mL of groundwater. This slurry had initial concentrations of 70 mg/L of PCE, 1 mg/L of TCE and 0.05 mg/L of cis-DCE, which was the result of existing soil contamination. The batch experiment was conducted in clear, 25 mL borosilicate glass ampules (Kimble-Kontes, Vineland, NJ). Prior to use, the ampules were autoclaved for 25 min at 121 °C, placed in a glass desiccator which was evacuated at 750 mm Hg for 1 h and then filled with argon gas. Within an argon-filled glove bag, 88 ampules were each loaded with ca. 20 mL of soil the clay slurry, temporarily sealed with aluminum foil, and then permanently flame-sealed by melting the top 1 cm of the ampule neck with a propane-oxygen torch.

Ampules were sequentially numbered during preparation and then incubated at 25 ± 1 °C in an enclosed container, 50 ± 3 °C and 70 ± 3 °C in separate water baths (Thermo Electron Corp., Marietta, OH), or 95 ± 3 °C in a custom-made enclosure that consisted of an insulated box fitted with 1/4-inch ID steel tubing. The steel tubing was connected to a constant-temperature

recirculation bath heater (Neslab RTE-211) filled with silicone oil. Twenty ampules were placed in the 25 °C container (4 ampules × 5 incubation times), while 20 ampules each were incubated in the 50 °C and 70 °C water bath, and the 95 °C insulated box (4 ampules × 5 incubation times). Ampules were removed from their controlled temperature environments after 11.9, 37.5, 75.0, 113.9, and 184.9 days of incubation time and cooled to 25 °C to facilitate destructive sample collection. The mass of PCE associated with the solid phase in each ampule, with exception of the 37.5 day ampules, was determined using a single solvent extraction step that consisted of adding 15 mL of methanol to each ampule after draining the free water, followed by transferring the soil-methanol slurry to a screw-top vial that was maintained at 25 ± 3 °C for at least 24 h prior to analyzing the methanol for PCE content. For the 37.5 days ampules, a series of methanol extractions was performed where the concentration of PCE in the 15 mL of methanol added to the soil from each ampule was determined after 3 days of equilibration time; the methanol was drained, and 15 mL of fresh methanol added.

3.4.6. Reactivity of TCE with Pemaco Soil

Soil samples were obtained from the Pemaco Superfund site described in Section 3.3.4. Ampules were prepared with vadose zone soil collected from Boring TMP-17 between 56 and 57 feet below ground surface. The soil contained little TCE (i.e., < 1 mg/kg) so before preparing ampules, the soil was humidified with water and then gas-phase TCE was added using the following procedure. The soil was removed from the acetate liner and transferred into a 4.8 cm diameter by 60 cm long chromatography column so that 1.6 kg filled the column. Then breathing grade air at 50 mL/min was passed through a 250 mL gas washing bottle filled with DI water and then into the column filled with Pemaco soil. After passing air saturated with water through the column for a period of 15 h, a second gas washing bottle filled with neat TCE was added so that air saturated with water and TCE vapor would pass through the soil filled column. After introducing 11.3 g of vapor phase TCE into the column so that the theoretical soil TCE concentration would be 6,840 mg/kg, the column was sealed and allowed to rest for 24 h.

The batch experiments were conducted in clear, 25 mL borosilicate glass ampules (Kimble-Kontes, Vineland, NJ). Prior to use, the ampules were rinsed with 18.1 MΩ/cm DI water, autoclaved for 25 min at 121 °C, and then allowed to air dry. Within a laminar flow hood, 81 ampules were loaded with 17 ± 5 g of soil, temporarily sealed with aluminum foil, and then permanently flame-sealed by melting the top 1 cm of the ampule neck with a propane-oxygen torch. No water was added to the ampules and ambient laboratory air constituted the gas phase as the soil was from the unsaturated zone.

Ampules were sequentially numbered during preparation and then incubated at 25 ± 1 °C in an enclosed container, 50 ± 3 °C and 70 ± 3 °C in separate water baths (Thermo Electron Corp., Marietta, OH), or 95 ± 3 °C in a custom-made enclosure that consisted of an insulated box fitted with 1/4-inch ID steel tubing. The steel tubing was connected to a constant-temperature recirculation bath heater (Neslab RTE-211) filled with silicone oil. Twenty ampules were placed in the 25 °C container (4 ampules × 5 incubation times), while 20 ampules each were incubated in the 50 °C and 70 °C water bath, and the 95 °C insulated box (4 ampules × 5 incubation times). Ampules were removed from their controlled temperature environments after 13.8, 39.8, 66.9, 92.9, and 118.9 days of incubation time and cooled to 25 °C to facilitate destructive sample collection. Due to the lack of water in the ampules, the gas sampling procedure was modified from the previous ampule experiments. Immediately after removing the ampule neck, a sheet of

parafilm was affixed to the opened ampule and gas samples were removed from the ampule using gas tight syringes equipped narrow gauge stainless steel needles. A 250 uL gas sample was removed and immediately injected into a mass spectrometer (MS) and a second 2 mL gas sample was removed for injection into a thermal conductivity detector (TCD). The mass of TCE, chloride, and organic acids associated with the solid phase in each ampule was determined using a single water extraction step that consisted of adding 10 mL of 18.1 MΩ/cm DI water to each ampule. After equilibrating for a period of at least 1 hour, a 1 mL aqueous sample was collected from the parafilm sealed ampule and transferred into a headspace vial for analysis. A subsequent 1 mL sample was collected for analysis by ion chromatography (IC).

3.4.7. Analytical Methods for Ampule Experiments

Aqueous phase concentrations of chlorinated ethenes in the ampules were determined using an Agilent Model 6890N GC equipped with a Teledyne-Tekmar HT3 headspace autosampler and a 30 m long by 0.25 mm OD DB-5ms column with 0.25 µm film thickness connected to an Agilent Model 5975 Mass Select Detector (MSD) or an Hewlett-Packard (HP) 6890 GC equipped with a 30 m long by 0.32 mm OD Agilent DB-5 column connected to an FID. The headspace autosampler was programmed to hold each sample at 70 °C for a period of 15 min prior to transferring 1 mL of headspace gas through heated silcosteel tubing to the GC inlet. The GC oven was initially at 35 °C for 7 minutes, then increased to 60°C at a rate of 10°C per minute, and held at 60 °C for 2 minutes. Calibration standards for vinyl chloride (C_2H_3Cl), *cis*-1,2-dichloroethylene, TCE, and PCE were prepared by injecting small volumes (< 10 µL) of methanol stock solution into 22 mL headspace vials filled with 1 mL of DI water.

The haloacetic acid (i.e., dicholoracetic acid) content of ampule water was determined using procedures based on EPA method 552.2 (US EPA, 1995c). This method involved: 1) pH adjustment, 2) liquid-liquid extraction, 3) derivatization, and 4) neutralization, followed by GC analysis. In addition, concentrations of formate ($CHOO^-$), glycolate (COH_3COO^-), acetate (CH_3COO^-), oxalate ($COOHCOO^-$), sulfate (SO_4^{2-}) and chloride (Cl^-) in the aqueous phase were determined using a Dionex DX-100 ion chromatograph (IC) equipped with an AS14A IonPac column. A 1 mL aqueous sample from selected ampules was injected into the IC at a flow rate of 1 mL/min using an eluent consisting of 8 mM Na_2CO_3 + 1 mM $NaHCO_3$. Organic acid and sulfate calibration standards were prepared from solids over a concentration range of 0.02 to 0.50 mM. Chloride concentrations were also determined using the colorimetric method of Bergmann and Sanik (1957). Chloride calibration standards, ranging in concentration from 0.02 to 1 mM, were prepared by serial dilution of a certified 1,000 mg/L chloride solution (SPEX CertiPrep, Metuchen, NJ). The dissolved oxygen content of water held in the ampule neck was measured immediately after opening using either the Rhodazine D or Indigo Carmen method (CHEMetrics, Calverton, VA).

Concentrations of carbon monoxide (CO) and carbon dioxide (CO_2) in gas phase of the ampules were determined using a HP 6890 GC equipped with a heated gas sampling valve with a 250 µL sample loop. The gas sample in the 250 µL loop was injected into a GC inlet operated in the splitless mode for 45 s at 200 °C. The sample passed through a 30 m by 0.32 mm OD Carboxen-1010 column (Supleco, Bellefonte, PA) connected to either a custom-made methanizer and an FID or a TCD. The methanizer was built by loading a 1/16-inch OD 316 stainless steel tubing with 100 mesh nickel powder and installing the tube into a cartridge heater. The methanizer was heated to 420 °C and hydrogen was used as the capillary column carrier gas for

the catalytic reduction of CO and CO_2 to methane (CH_4) by nickel within the methanizer. This technique achieved a detection limit of ca. 16 ppmv for CO and 37 ppmv for CO_2. With the TCD, detection limits increased to ca. 500 ppmv for CO and to 1,500 ppmv for CO_2. Gas standards were prepared from 99% grade ethene, ethane (Scotty Specialty Gases, Plumsteadville, PA) or acetylene (Airgas, Inc., Radnor, PA), while CO, CO_2, and methane standards were prepared from a certified mixture (Matheson Tri-Gas, Twinsburg, OH). The GC/methanizer/FID was calibrated using a serial dilution of certified gas mixture (Scotty Specialty Gases, Plumsteadville, PA) that contained carbon dioxide (15%), carbon monoxide (7%), oxygen (5%), and nitrogen (73%). Calibration standards were prepared using a 500 mL syringe (Hamilton Company, Reno, NV) with nitrogen as the dilution gas.

After analyzing 4 mL of the gas sample using the GC/methanizer/FID, approximately 1 mL of the gas sample was injected into the inlet of a Varian Star 3600 GC equipped with a 30 m long by 0.32 mm OD Varian CP-Sil 8ms column connected to a Saturn 2000 Mass Spectrometer (MS). Unknown compounds were identified using software (SaturnView ver. 5.41, Varian, Palo Alto, CA) that matched mass spectra to compounds in the NIST/EPA/NIH Mass Spectral Library (NIST98). The mass spectrometer was automatically tuned by adjusting the electron multiplier voltage to achieve a gain of 1×10^5 electrons per ion and by mass axis calibration to an internal reference compound (perfluorotributylamine) prior to each use.

The concentration of ferrous iron (Fe^{2+}) and total iron ($Fe^{2+} + Fe^{3+}$) was determined using the ferrozine method as developed by Stookey (1970) and Viollier et al. (2000). The ferrozine method involved transferring a 50 µL aqueous phase sample from each ampule into a polystyrene cuvette containing 300 µL of ferrozine reagent (Reagent A) consisting of 0.01 M ferrozine in 0.1 M ammonium acetate. After 2 minutes, 2.5 mL of DI water was added to the cuvette and, after an additional 8 minute period, the absorbance at 562 nm was measured using a Cary 3E Spectrophotometer (Varian, Palo Alto, CA). This initial reading represented the amount of Fe^{2+} present in the sample. After taking the 562 nm reading, 200 µL of a second reducing reagent (Reagent B), consisting of 1.4M hydroxylamine hydrochloride in 2M HCl, was added to the cuvette. After waiting 8 minutes, 50 µL of a third reagent (Reagent C), 10M ammonium acetate pH 9.5 buffer, was added and then, after an additional 2 minutes, the absorbance at 562 nm was measured representing the total iron ($Fe^{2+} + Fe^{3+}$) in the sample. The ferrozine method was calibrated using a freshly prepared aqueous solution of ferrous ammonium sulfate at the concentrations of 4, 12, 28, and 40 mg/L.

The concentration of sulfide (S^{-2}) was determined using the methylene blue method (Haddad and Heckenberg, 1988), which involved adding 1 mL of ampule water to a 2 mL vial followed by 50 µL of a 0.1 M methylene blue in 1M sulfuric acid solution and then 1 drop of a saturated ferric chloride solution. The vial was capped with a Teflon®-lined crimp and inverted once. After allowing the sulfide to react with methylene blue, 0.5 mL of the solution from the 2 mL autosampler vial was added to 2.5 mL of DI water in a quartz cuvette and the light adsorption at a wavelength of 664 nm was recorded. The methylene blue method was calibrated using a solution of sodium sulfide in N_2-sparged DI water at concentrations of 1, 10, and 100 mg/L.

The dissolved oxygen (DO) content of water held in the ampule neck was measured immediately after opening using a self-filling CHEMets ampule (CHEMetrics, Calverton, VA). Either the Rhodazine D method (part# K7501) was used for the 0 to 1 mg/L DO range or Indigo Carmen method (part# K7512) was used for the 1 to 10 mg/L DO range.

A sample of acid-treated Ottawa sand was sent to the U.S. Silica Co. Laboratory in Berkeley Springs, WV for X-ray diffraction (XRD) analysis. The mineral phases identified in the sand included pyrite, marcasite (FeS_2 - polymorph of pyrite), and hematite (Fe_2O_3). The Ottawa sand was also analyzed for 20 elements using inductively coupled plasma mass spectrometry (ICP-MS) (Thermo Jarrell-Ash, Enviro 36) after a double acid digest by the Chemical Analysis Laboratory at The University of Georgia located in Athens, GA.

3.5. Fate of PCE and TCE in Heated VOA Vials

An experiment was performed to determine if volatile organic analysis (VOA) vials were suitable for the study of aqueous-phase dechlorination reactions at elevated temperatures. Volatile organic analysis vials, made from clear borosilicate glass with average volume of 43 mL, were obtained from EP Scientific Products (Miami, OK). The vials were sealed with open-top polypropylene screw-caps fitted with 3.175 mm thick Teflon®-lined silicone septa. The vials were certified clean and used as delivered. The aqueous stock for the VOA vial experiment was prepared using DI water that was purified with a Milli-Q system (Gradient A10, Millipore Corp., Billerica, MA) to an electrical resistance of 18.2 MΩ/cm and a total organic carbon content of less than 2 μg/L. The Milli-Q DI water was sparged with N_2 gas for 45 min until the dissolved oxygen concentration was less than 0.4 mg/L based on the Rhodazine D method (CHEMetrics, Inc. Calverton, VA). Each of the 72 VOA vials were filled to capacity with de-oxygenated Milli-Q DI water and temporarily sealed with a screw-cap. PCE or TCE was added to each vial by removing the screw-cap and injecting a 20 μL aliquot of methanol stock. The PCE-methanol stock had a concentration of 0.024 mg/μL resulting in an initial VOA vial concentration of 11.1 mg/L, while the TCE-methanol stock had a concentration of 0.020 mg/μL resulting in an initial VOA vial concentration of 9.2 mg/L. After adding the solvent spike, each vial was immediately sealed so that no gas bubbles were present to achieve a zero headspace aqueous-phase system. Of the 72 vials prepared, 36 contained PCE and 36 contained TCE, of which half (18 vials) were placed in a dark cabinet maintained at 21 ± 3 °C, and 18 were placed in a water bath maintained at 55 ± 3 °C. The vials incubated in the water bath were completely submerged so that the heated bath water covered the septa during incubation.

Six VOA vials were collected from each temperature subset at selected times, allowed to reach room temperature, and the contents of each vial was sampled to determine the compounds present. A 1 mL aqueous sample was obtained from each VOA vial by piercing the vial septa with a needle affixed to a gas tight syringe, removing 1 mL of aqueous sample, which was transferred to a headspace vial and then analyzed using an automated headspace sampler connected to a GC-MSD. After collecting the headspace sample, an additional 1 mL sample was collected and injection into an ion chromatograph to determine the anions present.

3.5.1. Analytical Methods

Aqueous phase concentrations of PCE and TCE in the VOA vials and TCE in the flame-sealed ampules were determined using an Agilent Model 6890N GC equipped with a Teledyne-Tekmar HT3 headspace autosampler and a 30 m long × 0.25 mm OD DB-5ms column with 0.25 μm film thickness connected to an Agilent Model 5975 MSD. The headspace autosampler was programmed to hold each sample at 70 °C for a period of 15 min prior to transferring 1 mL of headspace gas through heated silcosteel tubing to the GC inlet. The GC oven was initially at 35 °C for 7 minutes, then increased to 60 °C at a rate of 10 °C per minute, and held at 60 °C for 2 minutes. Calibration standards for vinyl chloride (C_2H_3Cl), cis-1,2-dichloroethylene, TCE, and

PCE were prepared by injecting small volumes (< 10 μL) of methanol stock solution into 22 mL headspace vials filled with 1 mL of DI water.

Concentrations of formate (CHOO⁻), glycolate (COH₃COO⁻), acetate (CH₃COO⁻), oxalate (COOHCOO⁻), sulfate (SO₄²⁻) and chloride (Cl⁻) in the aqueous phase were determined using a Dionex DX-500 IC equipped with an AS4A IonPac column. A 1 mL aqueous sample from each ampule was injected into the IC at a flow rate of 1 mL/min using an eluent consisting of 8 mM Na_2CO_3 and 1 mM $NaHCO_3$. Calibration standards, in the range from 0.01 to 1 mM, for formate and glycolate were prepared from 99% grade solids (ACROS Organics, Morris Plains, NJ), while acetate, oxalate, and sulfate solutions were prepared from ACS certified solids (Fisher Scientific, Fair Lawn, NJ). Chloride calibration standards were prepared by serial dilution of a certified 1,000 mg/L chloride solution (SPEX CertiPrep, Metuchen, NJ).

3.6. ELECTRICAL RESISTIVE HEATING (ERH) CELL

To evaluate the destruction and recovery of chloroethenes during electrical resistive heating of field soils, a laboratory-scale treatment system was constructed from a glass chromatography column. The treatment cell consisted of a 5-cm ID × 90 cm long glass chromatography column with total interior volume of 1.8 L (Figure 3-7). The treatment cell was assembled by loading 60 cm of the treatment cell with clean Federal Fine sand (US Silica, Berkeley, WV) followed by 30 cm of Great Lakes soil collected from Borehole F3 between 8 and 10 feet bgs. After filling the column with solids, two stainless-steel electrodes, consisting of standard 1/8-inch OD threaded stock (McMaster-Carr, Atlanta, GA), were then threaded through the nylon endplate and into the Great Lakes soil along with an electrically insulated K-type thermocouple (OMEGA Engineering Inc., Stamford, CT). Once assembled, groundwater collected from the Great Lakes site was pumped into the column at 0.5 mL/min to saturate the column.
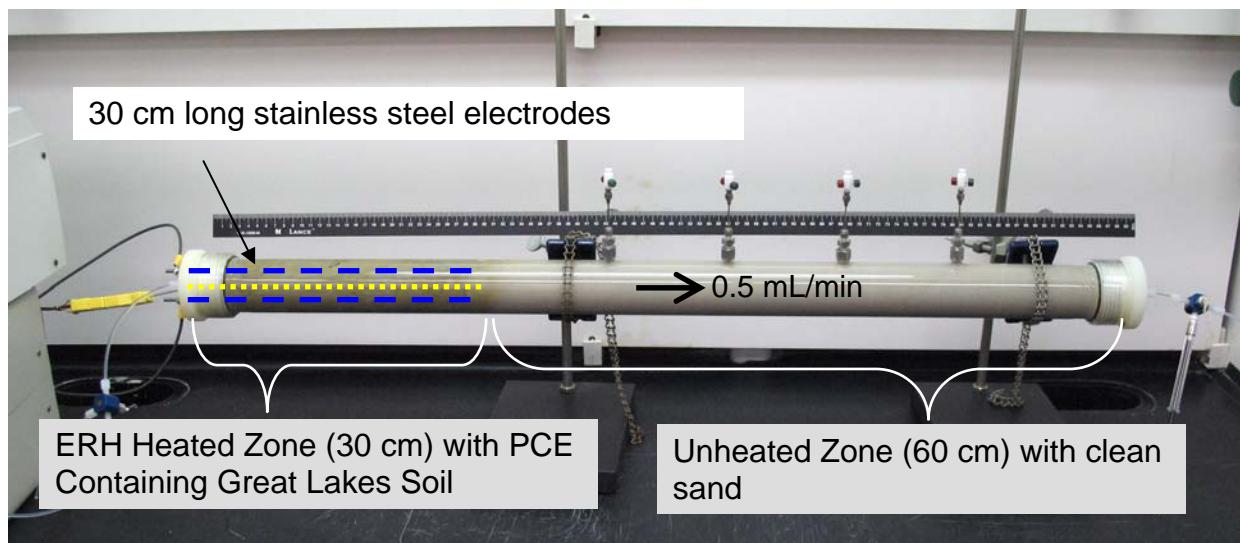
Figure 3-7. Electrical resistive heating (ERH) treatment cell experimental apparatus.

The column was custom fitted with four side ports through which 1/8-inch OD × 6 cm long nickel plated needles were placed to collect samples from the column interior. These side ports were located in the unheated, clean-sand portion of the column to monitor the chlorinated ethenes and anions coming from the Great Lakes soil before and during thermal treatment. The needles were sealed using mininert values (VICI Valco Instrument Co. Inc., Houston, TX) between sample collection events. After passing ca. 1.8 L of groundwater through the unheated column to establish baseline PCE concentrations, 120V AC power was applied to the two stainless steel electrodes using a Watlow Series 965 temperature feedback controller (Watlow Electric Manufacturing Co., St. Louis, MO). Samples were collected from the side ports and column effluent to track changes in chlorinated ethene and anion concentrations.

3.6.1. Analytical Methods

Aqueous-phase concentrations of chloroethenes were determined using an HP 6890 GC equipped with a Teledyne-Tekmar (Teledyne Technologies, Inc., Mason, OH) HT3 headspace autosampler and a 30 m long by 0.32 mm OD Agilent DB-5 column connected to an FID. The headspace autosampler was programmed to hold each sample at 70 °C for a period of 20 min prior to transferring the headspace gas to the GC injection port through silcosteel tubing heated to 140 °C. Calibration standards were prepared by injecting small volumes of a 10,000 mg/L chloroethene-methanol stock solution into 22 mL headspace vials filled with 1 mL of DI water.

Concentrations of carbon monoxide and carbon dioxide in gas phase of the ampules were determined using a HP 6890 GC equipped with a heated gas sampling valve with a 250 μL sample loop. The gas sample in the 250 μL loop was injected into a GC inlet operated in the splitless mode for 45 s at 200 °C. The sample passed through a 30 m × 0.32 mm OD Carboxen-1010 column (Supleco, Bellefonte, PA) connected to a TCD. Gas standards were prepared from 99% grade ethene, ethane (Scotty Specialty Gases, Plumsteadville, PA) or acetylene (Airgas, Inc., Radnor, PA), while CO, CO_2, and methane standards were prepared from a certified mixture (Matheson Tri-Gas, Twinsburg, OH). Calibration standards were prepared using a 500 mL syringe (Hamilton Company, Reno, NV) with nitrogen as the dilution gas.

Concentrations of formate ($CHOO^-$), glycolate (COH_3COO^-), acetate (CH_3COO^-), oxalate ($COOHCOO^-$), sulfate (SO_4^{2-}) and chloride (Cl^-) in the aqueous phase were determined using a Dionex (Sunnyvale, CA) IC equipped with a Dionex AS14 IonPac column. A 1 mL aqueous sample from each ampule was injected into the IC at a flow rate of 1 mL/min using an eluent consisting of 8 mM Na_2CO_3 and 1 mM $NaHCO_3$. Calibration standards, in the range from 0.01 to 1 mM, for formate and glycolate were prepared from 99% grade solids (ACROS Organics, Morris Plains, NJ), while acetate, oxalate, and sulfate solutions were prepared from ACS certified solids (Fisher Scientific, Fair Lawn, NJ). Chloride calibration standards were prepared by serial dilution of a certified 1,000 mg/L chloride solution (SPEX CertiPrep, Metuchen, NJ).

3.7. PCE AND TCE REACTIVITY IN A QUARTZ TUBE

The quartz tube experimental system consisted of a quartz-glass tube, a quartz-glass pre-mix chamber, and a quartz-glass effluent transition (Figure 3-8). The quartz tube was General Electric Type 124 fused quartz glass (Technical Glass Products, Mentor, OH), with an OD of 38 mm, wall thickness of 2 mm, and a length of 53 cm. The pre-mix chamber was custom made (Lillie Glassware, Marietta, GA) to provide an approximate 70 mL volume where gas and TCE could mix before entering the quartz tube. The effluent transition was custom made (Lillie

Glassware, Marietta, GA) from quartz glass to transition the gas flow from the 38 mm OD tube down to an 8 mm OD tube. The pre-mix chamber and effluent transition were connected to the quartz tube using custom made 38 mm ID, 316 grade stainless steel (316-SS) adapters (Swagelok Co., Salon, OH) fitted with Viton® O-rings.
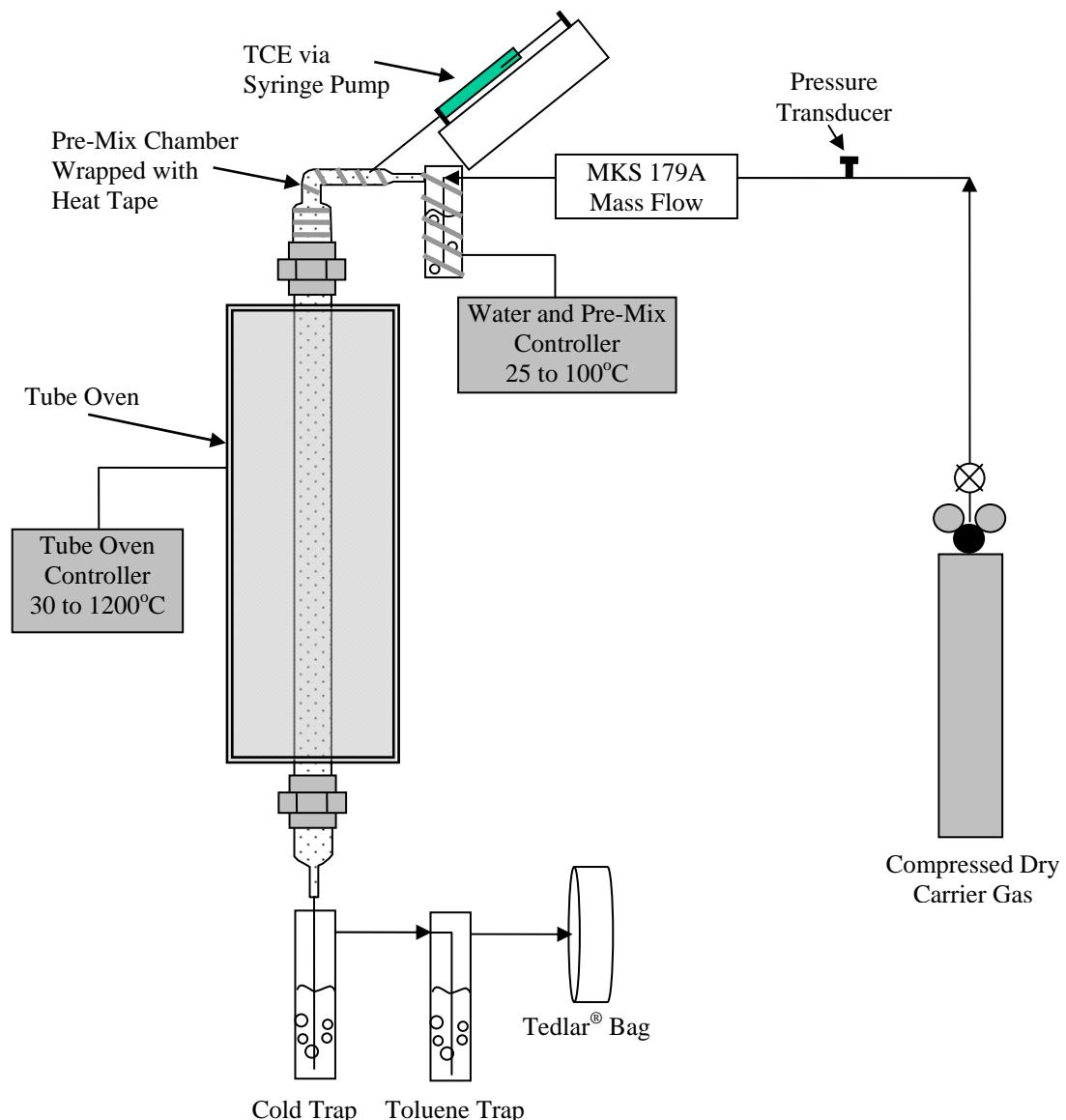
Figure 3-8. Quartz tube experimental apparatus.

The flow rate of the TCE-free carrier gas at 25 °C entering into the experimental apparatus was determined using a mass flow meter (Model 179A, MKS Instruments, Andover, MA). The mass flow meter was calibrated using an ADM2000 gas flow meter (J&W Scientific, Folsom, CA) that had been calibrated by California Integrated Coordinators (Placerville, CA). The pressure within the reaction system was determined using a pressure transducer (Honeywell, Freeport, IL), which was calibrated using a combination of water (0.02 to 0.05 bar) and mercury

(0.07 to 0.7 bar) filled manometers. Both the mass flow meter and pressure transducer were connected to a data logger (CR23X, Campbell Scientific, Logan UT) to automatically record the gas flow rate and pressure within the quartz tube system at one second intervals during each isothermal experiment.

The quartz tube and associated connectors were prepared prior to each isothermal experiment by washing in hot (45 ^{o}C) tap water with detergent (Versa-Clean, Fisher Scientific). The tube and connectors were then rinsed in deionized (DI) water and placed in a drying oven at 200 ^{o}C for a period of 2 h. The tube and connectors were allowed to cool to room temperature and the experimental apparatus was assembled and rinsed with approximately 20 mL of dichloromethane (DCM) for a period of 5 minutes. A 2 mL sample of the DCM rinse was collected and stored at 4 ^{o}C until analyzed to demonstrate the organic-free initial experimental condition. After collecting the DCM rinse, the experimental apparatus was disassembled and remained in the vent hood for a period of 5 minutes to remove the residual DCM. The apparatus was then re-assembled after placing the quartz tube within a 20 inch long $\times$ 1.5 inch ID galvanized steel pipe that was located in the tube oven (Model 21100, Barnstead-Thermolyne, Dubuque, IA). The steel pipe served to protect the tube oven from damage by shards of quartz glass that formed during quartz tube failures.

TCE was introduced into the pre-mix chamber, as shown in Figure 3-8, as neat liquid TCE at a fixed rate of 0.68 mL/h using a syringe pump (Model 11, Harvard Apparatus, Holliston, MA). This allowed the rate of TCE introduction to be fixed while adjusting the amount of water entering the quartz tube to vary the chlorine to hydrogen ratio inside the heated quartz tube. The process of introducing TCE into the pre-mix chamber consisted of initially recording the weight of a 1 mL gas-tight syringe, which contained approximately 0.34 mL (~0.5 g) of neat TCE, using an analytical balance (Model# AG245, Mettler-Toledo, Columbus, OH) with 0.001 g readability. The analytical balance had been checked using an ASTM E617 Class 2 certified traceable 20±0.0001 g weight (Cat.# 820000.2, Denver Instruments, Denver, CO) prior to determining the syringe weight. The syringe needle was then inserted through a Teflon$^®$-lined septum affixed with a crimp seal to a port located on the pre-mix chamber and TCE was injected at the slow rate of 0.68 mL/h for a period of 30 min. There were no drops of neat TCE visible at the syringe needle tip, which was located inside the premix chamber, when using this TCE injection rate so that TCE entered the quartz tube in the gas phase. After the 30 min TCE introduction period, the syringe was removed from the pre-mix chamber and the final weight recorded using the analytical balance and the amount of TCE introduced into the apparatus was determined by the difference in weight between the initial TCE-filled syringe and the final syringe weight after the 30 min TCE injection period. The quartz tube was then flushed with TCE-free humidified carrier gas for 45 min after removing the syringe to recover as much of the TCE introduced into the experimental apparatus as possible.

The carrier gases used were ultra zero grade air (UZA, Airgas-South, Inc., Marietta, GA) and was used as received. The carrier gas was humidified by passing through a mini-bubbler (ACE Glass, Vineland, NJ) filled with approximately 30 mL of DI water prior to entering the quartz tube (Figure 3-8). The DI water was freshly dispensed from a Nanopure$^®$ analytical deionization system (model D4741, Barnstead International, Dubuque, IA) with a conductance of greater than 18 MΩ/cm. The amount of water vapor entering the quartz tube was adjusted by increasing the temperature of the mini-bubbler and pre-mix chamber using a resistant-wire based heat tape (McMaster-Carr, Atlanta, GA) that was wrapped around the mini-bubbler and pre-mix

chamber and connected to a feedback voltage controller equipped with a K-type thermocouple. Three inlet temperatures were evaluated; 25, 60 and 100 °C. The temperatures were chosen to explore a range of chloride to hydrogen (Cl/H) ratios. The room temperature inlet condition had a calculated Cl/H ratio of 1 at a carrier-gas flow rate of 85 mL/min with the TCE liquid influent rate fixed at 0.68 mL/h. The 60 °C inlet temperature had a calculated Cl/H ratio of 0.28 and the 100 °C inlet temperature had a Cl/H ratio of 0.07. The 100 °C inlet temperature represented a condition where the number of hydrogen atoms in water was approximately 15 times greater than the number of chlorine atoms in TCE.

The carrier gas leaving the quartz-tube reactor passed through a two-stage cold-trap that was connected to a 40 mL vial filled with toluene in effort to trap phosgene for direct analysis on a GC equipped with an electron capture detector (ECD) (Figure 3-8). The cold trap facilitated using toluene as a trap fluid by isolating the potentially explosive toluene vapors from the heated quartz tube. The cold-trap consisted of two stages: the first stage was maintained at -10 °C and the second stage at -78 °C. The first stage of the cold trap consisted of a 10 cm long by 1 cm ID jacketed short-path condenser that was cooled to -10 °C by pumping a 1:1 (by volume) mixture of water and ethylene glycol through the jacket using a recirculation bath. The second stage of the cold trap was a 150 mL Erlenmeyer vacuum flask with aspirator that was place in an insulated container filled with crushed dry ice. The temperature within the Erlenmeyer flask was -78 °C, measured by a K-type thermocouple placed into the flask after 15 minutes of cooling. This two stage cold-trap was expected to retain compounds with a melting temperature of greater than -78 °C such as chloroform, carbon tetrachloride (CCl_4), and PCE (Table 3-6). Phosgene was expected to pass through the cold trap necessitating additional collection measures. A 40 mL vial was filled with approximately 30 mL of toluene (Optima grade, Fisher Scientific, Fair Lawn, NJ) and was connected to the aspirator nipple on the Erlenmeyer flask via 1/8-inch OD Teflon® tubing so that gas leaving the cold trap bubbled through the toluene before being captured in a 1.6L Tedlar® bag. Toluene was chosen since phosgene is known to be soluble in toluene (Ryan et al., 1996) and because a phosgene calibration standard prepared in toluene was obtained from Sigma-Aldrich, Inc. (Milwaukee, WI).

The compounds condensed within the two-stage cold trap were collected by repeatedly rinsing the condenser and Erlenmeyer flask with fresh aliquots of toluene. The process began by disconnecting the cold trap from the quartz tube and pouring approximately 30 mL of toluene through the condenser while connected to the Erlenmeyer flask. The condenser opening was then sealed with a glass ground-joint stopper and allowed to reach room temperature (25 °C). The external 40 mL toluene trap remained attached to the cold-trap to collect any compounds that were volatilized during the warming process. After reaching room temperature the toluene in the cold-trap was collected in a 100 mL volumetric flask and the cold-trap was rinsed three additional times with fresh toluene, which was also collected in the 100 mL flask. Samples were then collected from the 100 mL flask after filling to the volumetric mark and placed in 2 mL autosampler vials for analysis by GC methods. Separate samples were collected from the 40 mL toluene trap.

Table 3-6. Melting and Boiling Point Temperatures for Select TCE Degradation Products (data from Linstrom and Mallard, 2005).

Compound	Melting Temperature (°C)	Boiling Temperature (°C)
Phosgene	−128	8
Dichloroacetylene	−126	NA
toluene	−95	111
TCE	−85	87
Chloroform	−63	61
CCl₄	−23	77
PCE	−23	121
Hexachlorobutene	11	NA
Hexachloroethane	185	185 (sublimes)
Dichloroacetic acid	11	194
Hexachlorobutadiene	NA	215
hexachlorobenzene	227	325

NA – not available

3.7.1. Analytical Methods

The gas stream leaving the toluene trap was collected in 1.6 L Tedlar® bags to retain all single-carbon, non-condensable degradation products (e.g., carbon dioxide). Each bag was flushed three times with nitrogen gas prior to use. The Tedlar® bag was removed from the quartz tube effluent stream when full and a gas sample from the bag was immediately analyzed to determine the amount of CO and CO_2 formed by the degradation of TCE in the heated quartz tube. The gas sample from the Tedlar® bag was collected by pulling approximately 60 mL of the Tedlar® bag contents through a 250 μL gas sample loop attached to a gas sampling valve heated to 120 °C and located within an insulated box on an HP 6890 GC. The gas sample in the 250 μL sample loop was then injected into the GC inlet that was operated at 8.90 psi in the splitless mode for 0.75 minutes at 200 °C and was connected to a 30 m × 0.32 mm OD Carboxen-1010 column (Supelco, Bellefonte, PA) attached to a TCD. Helium was used as the capillary column carrier-gas at a constant flow of 2 mL/min and the GC oven was operated at 35 °C for 7 min followed by a 40 °C/min temperature ramp to 130 °C for 5 min. The TCD was operated at 210 °C with a helium reference flow of 15 mL/min and helium makeup flow at 5 mL/min. The Carboxen-1010 column is capable of separating O_2, N_2, CO, CO_2, and water. However, TCE and other organic compounds are retained within the carbon molecular sieve based column; the column was periodically conditioned at 200 °C to remove organic compounds. The GC-TCD was calibrated using serial dilution of an initial 100 mL volume of certified carbon dioxide (15%), carbon monoxide (7%), oxygen (5%), and nitrogen (73%) gas mixture (Scotty Specialty Gases, Plumsteadville, PA). The serial dilution was performed in a 500 mL syringe (Model S-500, Hamilton Company, Reno, NV) with nitrogen as the dilution gas. At least three CO/CO_2

concentrations were used to calibrate the GC-TCD response. This technique had a detection limit of approximately 300 µL/L (ppmv) for CO and 500 µL/L (ppmv) CO_2.

The GC-MS analysis of the DCM trap fluids from the 420 °C experiments identified a number of TCE degradation products. However, the amount of chloroform ($CHCl_3$), carbon tetrachloride (CCl_4), tetrachloroethylene (PCE), hexachloroethane (C_2Cl_6), hexachlorobutadiene (C_4Cl_6), and hexachlorobenzene (C_6Cl_6) were determined by GC-FID analysis. Master stock solutions (10,000 mg/L) for each of the previous compounds were prepared in DCM using ACS grade reagents (Sigma-Aldrich, Milwaukee, WI). Hexachlorobenzene (HCB) master stock was prepared by adding HCB solids to iso-octane. The GC-FID response was determined for each compound using at least four calibration standards prepared by volumetric dilution of the master stock at concentrations in the expected range.

The quartz tube was removed from the tube oven after cooling to room temperature and the interior of the sealed apparatus was rinsed with DI water and by toluene to determine the TCE degradation products that had formed and condensed onto the quartz glass surfaces. The apparatus was initially rinsed with approximately 30 mL of DI water for a period of 5 min to collect the water-soluble compounds (i.e., chloride) that formed at each experimental temperature. The second rinse used 30 mL of toluene for a period of 5 min to collect the non-polar TCE degradation products (i.e., hexachlorobenzene) from the experimental apparatus.

The haloacetic acid content of water samples was determined using procedures based on EPA method 552.2 (U.S. EPA, 1995c). This procedure involved 1) pH adjustment, 2) liquid-liquid extraction, 3) derivatization, and 4) neutralization followed by GC analysis. The water-rinse samples were contained in 40 mL glass vials sealed with Teflon lined septa affixed with screw caps. The pH of each water rinse sample was adjusted to less than 0.5 by adding 1.5 mL of concentrated sulfuric acid (H_2SO_4) to convert any carboxylates present into the acid form. The pH adjustment was followed by adding 5 mL of methyl-tert butyl ether (MTBE) to the 40 mL vials which were then resealed and hand shaken for 2 min to extract the haloacetic acids. Approximately 3 mL of the MTBE was then transferred from each 40 mL vial to 14 mL glass vials using a Pasteur pipette. One mL of acidic methanol (10% H_2SO_4) was added to each 14 mL vial, which were sealed with a Teflon®-lined septa affixed with a screw cap and then placed in an oven at 50 °C for a period of 2 h to convert the carboxylic acids to their derivatized, methyl ester form. After cooling the 14 mL vials to room temperature, the MTBE extract and acid methanol mixture was neutralized by adding 2 mL of saturated sodium bicarbonate solution. Two, 1 mL samples of the MTBE extracts were transferred from the 14 mL vials into 2 mL autosampler vials and the internal standard (1,2,3-trichloropropane) was added to each 2 mL vial, and the vials were then sealed with Teflon®-lined septa affixed with aluminum crimps.

The analysis of the MTBE extracts consisted of using an HP 7683 automatic liquid sampler to inject 1 uL of sample into an HP 6890 GC equipped with a 30 m × 0.32 mm OD HP-1 capillary column connected to an ECD. The GC inlet was operated at 7.00 psi in the splitless mode for 0.5 min at 200 °C with helium as the column carrier gas at a constant flowrate of 2 mL/min. The GC oven was operated at 35 °C for 21 min followed by an 11 °C/min temperature ramp to 136 °C for 3 min, and a final temperature ramp of 20 °C/min to 230 °C for 3 min. The ECD was operated at 250 °C with a N_2 gas makeup flow of 60 mL/min. Dichloroacetic acid calibration standards at concentrations of 12, 50, 100, and 400 ug/L were prepared from a 60 mg/L primary dilution standard made from ACS grade dichloroacetic acid (Sigma-Aldrich, Milwaukee, WI). The calibration samples were processed with each sample batch along with at

least two uncontaminated water samples including freshly dispensed DI water and a storage blank.

The GC-ECD chromatograms from the analysis of the MTBE extracts collected from the 420 ^{o}C UZA experiments contained peaks that eluted at times different than dichloroacetic acid. An HP 6890 GC equipped with a 30 m × 0.32 mm DB-5ms column connected to an HP 5973 MSD was used to identify the compounds associated with the unknown peaks. The GC operating conditions were identical to the GC-ECD method given above. Compounds were identified using software (ChemStation ver. D.00.00.38, Agilent Technologies, Palo Alto, CA) that matched their mass spectra with reference mass spectra in the NIST/EPA/NIH Mass Spectral Library (NIST02).

3.8. SPORE FORMATION IN RESPONSE TO HEATING

The initial goal of this study was to explore the spore-formation capability of the known PCE-to-*cis*-DCE-dechlorinating *Clostridium* isolate, *Clostridium bifermentans* strain DPH-1. However, based on the results of initial experiments, additional experiments were also conducted to characterize the novel PCE-dechlorinating isolate, *Desulfitobacterium hafniense* JH1.

3.8.1. Culture and Medium Preparation

Culture DPH-1 was obtained from K. Takamizawa and was maintained in 160 mL (nominal capacity) serum bottles containing 100 mL anoxic, reduced, bicarbonate-buffered (30 mM) mineral salts medium (Sung et al., 2006a) amended with acetate (5 mM), citrate (5 mM), yeast extract (2 g/L), and PCE (240 μM, aqueous concentration). All cultures received 3% (vol/vol) inocula and were incubated at 24 °C without agitation in the dark.

3.8.2. Resolution of Culture DPH-1 into Two Populations

In order to ensure culture purity, fluid from culture DPH-1 was spread on Luria Bertani agar plates inside an anoxic chamber (95% nitrogen/5% hydrogen, vol/vol). After colonies formed, inside the anoxic chamber, cells from isolated colonies were transferred to liquid medium using sterile pipette tips. In order to isolate the organism responsible for PCE dechlorination, two sequential dilution-to-extinction series were performed in 20-mL (nominal capacity) vials containing 9 mL of mineral salts medium amended with acetate (5 mM), H$_2$ (10% headspace volume), and PCE (2.5 μl) dissolved in hexadecane (47.5 μl) to yield an initial aqueous phase PCE concentration of approximately 460 μM (Löffler et al., 2005). The 10% (vol/vol) inoculum for the first dilution-to-extinction series was an actively dechlorinating liquid DPH-1 culture. The 10% (vol/vol) inoculum for the second dilution-to-extinction series was the 10^{-10} vial of the first dilution-to-extinction series.

Genomic DNA was extracted from both the non-dechlorinating isolate obtained following clonal purification on agar plates and from the dechlorinating isolate obtained from the dilution-to-extinction series. Using bacterial primers 8F and 1525R, 16S rRNA genes from both cultures were PCR-amplified, in separate reactions, as described (Ritalahti and Löffler, 2004). The 16S rRNA gene amplicons were cloned, and four cloned fragments from each PCR reaction was sequenced (Ritalahti and Löffler, 2004).

3.8.3. Physiological Characterization of Strain JH1

Electron acceptor utilization was tested in 60-mL (nominal capacity) serum bottles containing 30 mL anoxic, reduced, bicarbonate-buffered mineral salts medium amended with 5 mM pyruvate. Cultures were amended with undiluted chloroethanes, chloroethenes, chloromethanes, chloropropanes, or 2-chlorotoluene using a gas-tight Hamilton syringe (1800 series; Hamilton, Reno, NV) to yield final aqueous concentrations ranging from 100 to 250 µM. Chlorinated aliphatic compounds and 2-chlorotoluene were analyzed by GC as described (He et al., 2002). Hexachlorobenzene was added using a Hamilton syringe from a methanolic stock to an aqueous concentration of 0.09 µM and analyzed by liquid/liquid extraction in hexane, followed by gas chromatographic separation, and detection using an electron capture detector. 3-Chloro-4-hydroxybenzoate, nitrate, sulfate, and sulfite were added from anoxic, sterile, aqueous stock solutions using plastic syringes to final concentrations of 1 to 2 mM. 3-Chloro-4-hydroxybenzoate was analyzed as described (Wu et al., 2006) and inorganic anions were analyzed with a Dionex ICS-3000 IC equipped with an AS14 4-mm column (Dionex, Sunnyvale, CA). Soluble Fe(III) (as Fe(III) citrate) and poorly crystalline Fe(III) oxide were prepared as described (Sung et al., 2006a) and added at 5 mM (nominal) concentrations. Fe(II), total iron, ammonia, and sulfide concentrations were determined colorimetrically (Cline, 1969; Parsons et al., 1984).

To test electron donor utilization, culture vessels were amended with 100 µM PCE and inocula (3%, vol/vol) from a culture that had consumed all pyruvate. Once PCE dechlorination ceased due to electron donor limitation, cultures were amended with potential electron donors including acetate (5 mM), ethanol (170 µM), formate (5 mM), or H_2 (10% headspace volume). To test spore formation, strain JH1 cultures were exposed to temperatures ranging from 60 to 80 °C for 10 minutes as described previously (Griffin et al., 2004).

3.8.4. Identification of the PCE Reductive Dehalogenase Gene in Strain JH1

To identify the PCE reductive dehalogenase (rdase) gene in strain JH1, primers were designed both to amplify the *pceC* gene (GenBank accession number AJ277528) reported in culture DPH-1 (Okeke et al., 2001) and the *pceA* gene (accession number AP008230.1) reported in *Desulfitobacterium hafniense* Y51 (Suyama et al., 2002). Both *pceC* and *pceA* gene targeted primer pairs, (pceCF, 5'-CGGTCATCAGAGAAATAATG and pceCR, 5'-GCTGAAGTTTATAATAAAGA) and (pceAF, 5'-CGGACATCGTGGCTCCGAT and pceAR, 5'-CTTGTCCGGAGCAAGTTC), respectively, were designed based on published degenerate primers and gene sequences (Okeke et al., 2001; Suyama et al., 2002). In PCR reactions using both primer pairs, genomic DNA from the mixed DPH-1 culture, strain JH1, and the *Clostridium* isolate served as templates in separate PCR reactions. In the *pceC* gene targeted PCR, a range of annealing temperatures (32.6 to 41.6 ºC) and $MgCl_2$ concentrations (2.5 to 4.0 mM) were tested. In the *pceA* gene targeted PCR, reactions were carried out as described (Ritalahti and Löffler, 2004), but at an annealing temperature of 46.5 ºC. Amplicons were visualized in ethidium bromide-stained agarose gels and select amplicons were purified (Qiagen QIAquick PCR Purification Kit, Germantown, MD) and sequenced using primers pceAF and pceAR.

3.9. EFFECT OF ELEVATED TEMPERATURES ON CHARACTERIZED DECHLORINATING CULTURES

Experiments were conducted to determine how elevated temperatures effect reductive dechlorination activity and *Dehalococcoides* (*Dhc*.) biomarker quantification in characterized PCE-to-ethene dechlorinating populations. Further experiments determined if dechlorination activity can be recovered after cooling from incubation at elevated temperatures.

3.9.1. Cultures and Medium Preparation

Two mixed PCE-to-ethene dechlorinating cultures were used in this study, Bio-Dechlor Inoculum (BDI) and culture OW. BDI is a commercially available, non-methanogenic, PCE-to-ethene dechlorinating culture that has been successfully applied for bioremediation of field sites (Ritalahti et al., 2005). BDI contains three unique *Dhc*. strains, strain BAV1, strain GT, and strain FL2 (Ritalahti et al., 2006). Strain BAV1 is capable of metabolic reductive dechlorination of *cis*-DCE and VC (He et al., 2003) and contains the *bvcA* gene (Krajmalnik-Brown et al., 2004). Strain FL2 is capable of metabolic reduction of TCE and *cis*-DCE and contains the *tceA* gene (He et al., 2005). Strain GT is capable of metabolic reduction of TCE, *cis*-DCE, and VC and contains the *vcrA* gene (Sung et al., 2006b). BDI also contains a *Dehalobacter* population similar to the PCE-to-*cis*-DCE-dechlorinating *Dehalobacter restrictus* (Holliger et al., 1998). Culture OW is a methanogenic PCE-to-ethene dechlorinating culture that contains *Dhc*. populations containing the *tceA* and *vcrA* genes. Culture OW also contains *Dehalobacter*, *Geobacter*, and *Sulfurospirillum* populations similar to organisms known to reduce PCE-to-*cis*-DCE (Daprato et al., 2007).

Reduced, anaerobic, bicarbonate-buffered (30 mM) mineral salts medium was prepared as described (Amos et al., 2007) and cultures were grown in 160-ml (nominal volume) glass serum bottles containing approximately 100 ml of medium and a N_2/CO_2 (80% / 20%, vol/vol) headspace. Cultures were amended with 0.1 ml of PCE or TCE dissolved in methanol to final concentrations of less than 35 mg/L. In BDI and OW cultures 10 mM lactate and methanol served as electron donors, respectively, and were amended to cultures via sterile syringe from aqueous, anoxic stock solutions. Triplicate or duplicate cultures were incubated statically, upside-down, in the dark.

3.9.2. Experiments Assessing Reductive Dechlorination Activity at Elevated Temperatures

To determine how reductive dechlorination activity varies with incubation temperature, cultures were amended with PCE or TCE and incubated at temperatures ranging from 30 to 45 ℃. In experiments with the BDI culture, bottles containing anaerobic, sterile, mineral salts medium were inoculated (3%, vol/vol) with BDI and amended with PCE in methanol and incubated at 24 ℃. Once PCE was entirely converted to ethene, cultures were amended with PCE or TCE in methanol and placed at 30, 35, 40, and 45 ℃. In experiments with culture OW, bottles containing mineral salts medium were inoculated (5% vol/vol) with culture OW, amended with PCE, and immediately placed at 30, 35, 40, and 45 ℃. In experiments with both cultures, periodically, aqueous or gaseous samples were removed via sterile syringe for chlorinated ethene, acetate, or H_2 analysis or for DNA and RNA extraction. The removed volume was replaced either with sterile N_2 gas or with sterile mineral salts medium.

3.9.3. Experiments Assessing Recovery of Dechlorination Activity Following Cooling

To determine if VC reductive dechlorination activity can be recovered following incubation at elevated temperatures, chlorinated ethene concentrations were monitored in cultures following cooling from incubation at 40 ℃. Twelve serum bottles with mineral salts medium were inoculated with the BDI culture (3%, vol/vol) and cultures were amended with PCE in methanol. Once PCE was entirely converted to ethene, cultures were amended with TCE and immediately placed at 40 ℃. Triplicate cultures were removed from the 40 ℃ water bath and placed at room temperature (24 ℃) following 7, 14, 28, and 49 days of incubation in the water bath. Chlorinated ethene concentrations were measured periodically both during incubation at 40℃ and following cooling to 24 ℃ as described below. Aqueous volume removed for chloroethene analysis was replaced with an equal volume of sterile H_2.

3.9.4. DNA and RNA Extraction

Aqueous phase samples (5 ml) were removed from BDI cultures amended with PCE and incubated at 30, 35, and 40 ℃ and from cultures not amended with PCE and incubated at 30℃ immediately after amendment with PCE and after 6 and 42 days of incubation. Biomass was collected via centrifugation and RNA was stabilized using RNAprotect Bacteria Reagent (Qiagen, Valencia, CA) as described (Amos et al., 2008). Pellets were stored at -80 ℃ for no more than 2 months prior to DNA and RNA extraction using the Allprep DNA/RNA Mini Kit (Qiagen) as described (Amos et al., 2008). To remove DNA contamination from RNA, RNA was DNase treated with Baseline-Zero DNase (Epicentre) as per the manufacturer's instructions. After DNase treatment, RNA was purified using the RNeasy MinElute Cleanup Kit (Qiagen). The lack of an amplicon when RNA was used as the template in PCR reactions with primers targeting the universal bacterial16S rRNA gene demonstrated the removal of contaminating DNA. RNA was transcribed to cDNA using the SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen) as described (Amos et al., 2008).

3.9.5. Quantitative PCR

The *Dhc*. 16S rRNA gene and the *bvcA, tceA*, and *vcrA* reductive dehalogenase (rdase) genes were quantified using an ABI 7700 spectrofluorimetric thermal cycler as described (Amos et al., 2008). Because the *Dhc*. populations in the BDI culture contain both one copy of the 16S rRNA gene and one copy of either the *bvcA*, *tceA*, or *vcrA* genes, the summation of the number of *bvcA*, *tceA*, and *vcrA* genes should be equal to the number of *Dhc*. 16S rRNA genes. In fact, these values were approximately equal, verifying quantitative PCR (qPCR) results. Results from RNA extraction, reverse transcription, and cDNA qPCR are reported as transcript copies per cell normalized to positive control cultures. For example, the number of *bvcA* gene transcripts in cultures incubated at 35 ℃ is first divided by the number of BAV1 cells in cultures incubated at 35 ℃. Then, to normalize this value to the positive control culture, the number of *bvcA* transcripts per BAV1 cell in cultures incubated at 35 ℃ is divided by the number of *bvcA* gene transcripts per BAV1 cell in positive control cultures amended with PCE and incubated at 30 ℃. Similarly, 16S rRNA molecules per total *Dhc*. cells, *tceA* gene transcripts per FL2 cells, and *vcrA* gene transcripts per GT cells were all normalized to the same ratio in the positive control cultures.

3.9.6. Analytical Methods

Aqueous phase samples (1 ml) were collected to quantify chlorinated ethenes, ethene, and acetate as described (Amos et al., 2007). Gaseous phase samples (3 ml) were collected to quantify hydrogen concentrations. Hydrogen concentrations were measured using an HP 6890 GC equipped with a heated gas sampling valve, a 250 µL sample loop, and a 30 m × 0.32 mm OD Carboxen-1010 column (Supelco, Bellefonte, PA) connected to a TCD.

3.10. ELECTRON DONOR AVAILABILITY FOLLOWING THERMAL TREATMENT

Experiments were conducted to determine how thermal treatment affects the availability of electron donors for dechlorinating organisms. Chlorinated ethene concentrations were monitored in microcosms constructed with PCE-contaminated soil both during incubation at elevated temperatures (up to 95 °C) and following cooling. Furthermore, in order to determine if competitors to dechlorinators, such as methanogens, differentially impact electron donor availability based on previous incubation temperature, methane production was also quantified.

3.10.1 Site Description and Soil Preparation

Soil and groundwater samples impacted with PCE were collected from the Great Lakes Naval Training Center in Great Lakes, IL. Soil samples were collected from location F3 at between 8 to 10 feet bgs and groundwater samples were collected from wells MW06S and MW10S (a detailed description of the Great Lakes site is in section 3.3.3). Because the soil consisted of clay, a soil and groundwater slurry was prepared at room temperature (24 °C) by combining soil and groundwater in equal masses and breaking the clay into fragments in a disposable glove bag filled with ultra high purity argon. The clay slurry yielded a uniform solution with an aqueous phase PCE concentration of 132.5 mg/L and trace concentrations of TCE and *cis*-DCE.

3.10.2 Microcosm Construction, Incubation Conditions, and Sampling

At room temperature, (24 °C), 20 microcosms were constructed in an argon-filled glove bag. The soil and groundwater slurry was combined with 10 to 20 mL of sterile mineral salts medium (Amos et al., 2007) or site groundwater in 70-mL (nominal capacity) glass serum bottles to a total volume of 40 mL. Microcosms were capped with sterile black butyl-rubber stoppers prior to removal from the glove bag. Immediately following microcosm construction, five microcosms were autoclaved at 121 °C for 30 min to serve as abiotic controls. Triplicate microcosms and one abiotic control microcosm, were incubated at 24, 35, 50, 70, and 95 °C. The temperature in microcosms incubated at 35 and 50 °C was increased by 1 °C per day until reaching the target temperature. All other microcosms were placed at the target temperature immediately after construction. Following 4 months of incubation, microcosm temperatures were decreased by 5 °C per day to 24 °C. Microcosms were sampled periodically for chlorinated ethenes and gases, including methane, acetylene, and hydrogen. Both aqueous and gaseous samples were removed from microcosms using sterile syringes and needles. All the aqueous volumes removed were replaced with filter-sterilized groundwater or sterile medium and all gaseous volumes removed were replaced with sterile nitrogen gas.

3.10.3 Bioaugmentation and Biostimulation

After microcosms were cooled to 24 ºC, 10 mL of OW, a mixed, methanogenic, PCE-to-ethene-dechlorinating culture was added to each microcosm using a sterile, N_2-flushed syringe. Culture OW contains multiple *Dhc*. strains along with *Geobacter*, *Dehalobacter*, and *Sulfurospirillum* species capable of PCE-to-*cis*-DCE dechlorination (Daprato et al., 2007). Biostimulation was performed by adding 5 mL of sterile hydrogen gas via a sterile syringe to each microcosm.

3.10.4 Analytical Methods and Calculations

Chlorinated ethene and ethene concentrations were measured by headspace GC (Amos et al., 2007). Hydrogen, methane, and acetylene concentrations were measured using an HP 6890 GC equipped with a heated gas sampling valve, a 250 µL sample loop, and a 30 m × 0.32 mm OD Carboxen-1010 column (Supelco, Bellefonte, PA) connected to a TCD.

Concentrations of chlorinated ethenes, ethene, and methane were converted to molar values by accounting for partitioning between the aqueous and vapor phase using published temperature dependant Henry's constants (Staudinger and Roberts, 2001). To calculate the moles of electron equivalents consumed for reductive dechlorination, it was assumed that each dechlorination step required two electrons and therefore, the moles consumed were calculated according to the formula:

$$2(M_{TCE}) + 4(M_{DCEs}) + 6(M_{VC}) + 8(M_{ethene}) = M_{consumed} \tag{2-4}$$

where M_{TCE} is the number of moles of TCE, M_{DCEs} is the sum of the moles of *cis*- and *trans*-DCE, M_{vc} is the moles of vinyl chloride, M_{ethene} is the moles of ethene, and $M_{consumed}$ is the number of moles of electron equivalents consumed for reductive dechlorination. Dechlorination extent was determined by dividing the moles of electron equivalents used for reductive dechlorination at each time point by the moles of electron equivalents required for complete dechlorination. The number of electron equivalents used for methanogenesis was calculated by assuming that four moles of electron equivalents are required per mole of methane formed.

CHAPTER 4

RESULTS AND DISCUSSION

4.1. CONTAMINANT PHASE DISTRIBUTION

4.1.1. Henry's Law Constant for TCE as a Function of Temperature

The initial experimental trial (Trial 1) involved filling the 120 mL vessel with 50 mL of Milli-Q DI water that contained TCE at a concentration of 14.7 mg/L. The sealed vessel was then placed into a 5 L glass carboy filled with DI water and containing a Teflon-coated magnetic stir bar, which served as a constant temperature bath. The 5 L water bath and 120 mL vessel were placed on a bench top magnetic stirplate with heater and after mixing for 3 hours at 24 °C, three 1 mL water samples were collected from the 120 mL vessel and placed into separate headspace vials, which were analyzed for TCE using an automated headspace sampler and GC-MS. Three gas samples were collected from the 120 mL vessel 2 hours after collecting the water samples. The gas samples were injected into a GC-FID through an internal heated sample loop to determine the TCE concentration of the gas samples.

The resulting Henry's Law coefficient was 0.56 ± 0.11 at 24 °C, which is 48% greater than the value of 0.38 based on the correlation reported by Staudinger and Roberts (2001). The 48% difference was greater than the relative standard deviation (RSD) of the measurement replicates indicating that the value measured using the equilibrium system was significantly greater than the value reported by Staudinger and Roberts (2001). The temperature of the 5 L water bath was then increased and allowed to equilibrate at each temperature shown in Figure 4-1 for at least 3 hours before separate water and gas samples were collected and analyzed for TCE concentration. The equilibrium system remained sealed between each temperature and no volume of water or gas was introduced after each sample collection even. The Henry's coefficient values at each temperature, along with the measured aqueous (Cw) and gas (Cg) concentrations and the Henry's coefficients based on the correlation reported by Staudinger and Roberts (2001) are shown in Figure 4-1.
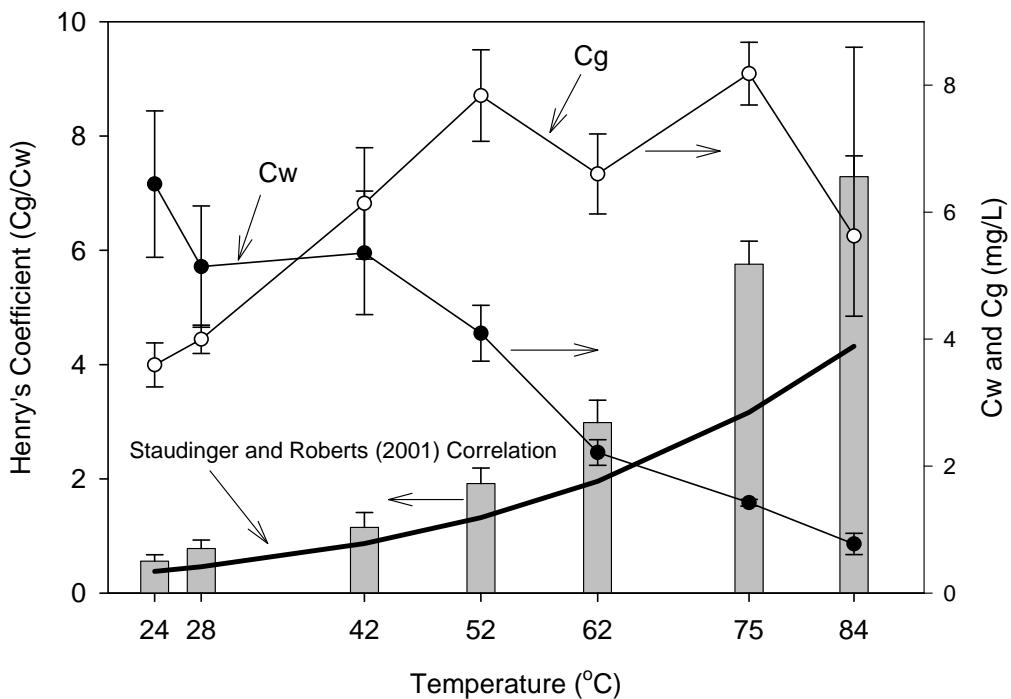
Figure 4-1. Results from Henry's Law experimental Trial 1 for TCE.

The Henry's Law coefficients increased with temperature during Trial 1. The increase in Henry's coefficient was primarily due to the decrease in aqueous phase concentration (Cw) at temperatures greater than 52 °C, while the gas phase concentrations (Cg) remained relatively consistent. The Henry's values determined during Trial 1 were more than 32% greater than the values reported in Staudinger and Roberts (2001).

A second experimental trial (Trial 2) was completed to determine if the initial results could be reproduced. Trial 2 was completed using the same experimental setup as in Trial 1, where the only difference was that 70 mL of TCE containing DI water stock was used instead of the 50 mL employed during Trial 1, and the equilibrium system was refilled with 70 mL of TCE-DI water stock between the 64 and 75 °C temperatures. The refill was required because the aqueous volume had been reduced to less than 30 mL due to successive sample collection events completed at 75 °C. The Henry's Law coefficients from Trial 2 were 11% less on average than those from Trial 1, with the exception of the values at 64 and 75 °C, which were 23 and 46% less than the corresponding values determined during Trial 1 (Figure 4-2).
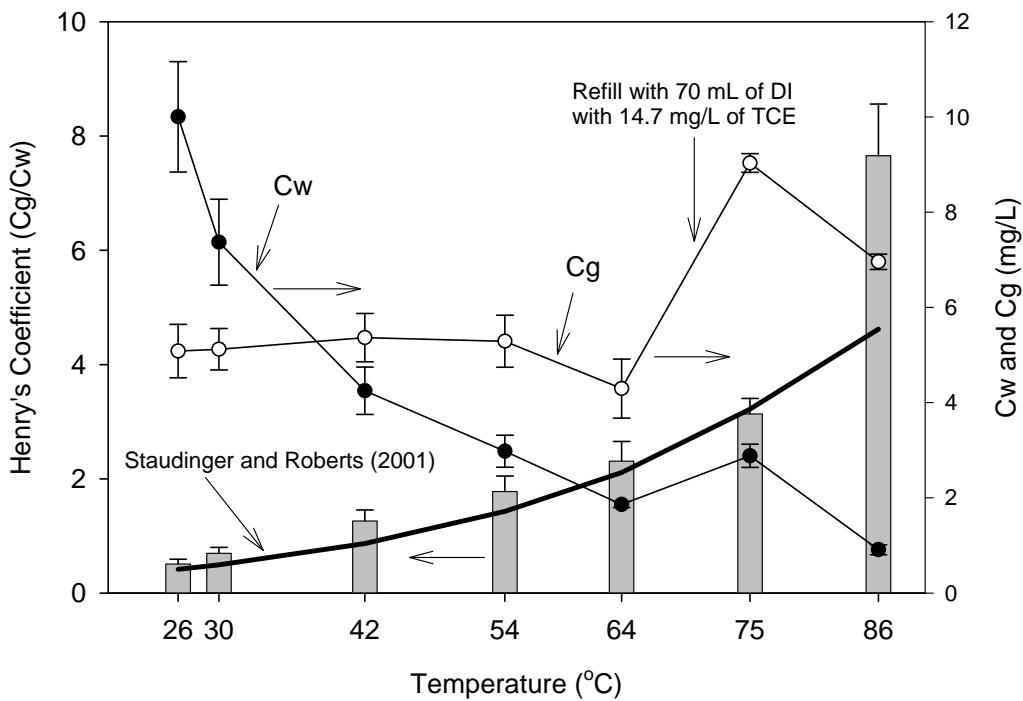
Figure 4-2. Results from Henry's Law experimental Trial 2 for TCE.

A third experimental trial (Trial 3) was completed after replacing the hot plate used to control the temperature of the DI water in the 5 L water bath with a recirculation heater capable of controlling the temperature to $\pm$ 0.01 °C (RTE 111, NESLAB Instruments, Inc., Portsmouth, NH). The Henry's Law coefficient from Trial 3 at 29.9 °C was 25% lower than the value from Trial 1 at 28 °C and 16% less than the value from Trial 2 at 29.5 °C. However, there was better agreement between Trials 2 and 3 at temperatures greater than 59.2 °C (Figure 4-3) compare to Trial 1.

The Henry's coefficient values determined during the three trials were at least 16% greater than the average values reported by Staudinger and Roberts (2001) in the ambient temperature range (i.e., 20 to 30 °C). The Staudinger and Roberts (2001) values represent the average from the results of twelve independent experiments completed between temperatures between 10 and 50 °C. Therefore, the equilibrium system over-predicted Henry's Law coefficients values established for ambient temperatures. The cause of this difference may have been due to the fact that the pressure within the equilibrium system decreased with each successive sampling event as no gas or liquid was added back into the vessel after removing samples. The use of a syringe to collect gas samples was suspect because the syringe needle was found to become blocked after penetrating the blue septa, and since the exact volume of gas collected was uncertain. To overcome these two concerns, a subsequent experimental trial (Trial 4) was completed with the tubing dedicated for the gas samples connected directly to the GC gas sample loop, and by allowing air to refill the vessel after completing each sample collection event to equilibrate the system to atmospheric pressure.
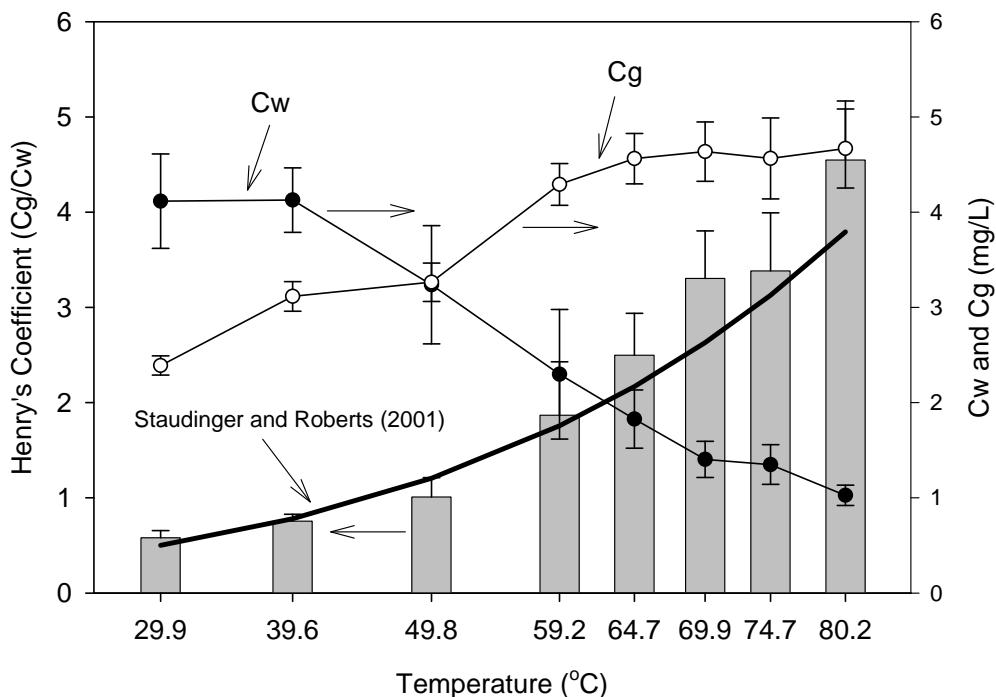
Figure 4-3. Results from Henry's Law experimental Trial 3 for TCE.

In addition to connecting the gas sample line directly to the GC gas-sample loop, there was one other change during Trial 4, which involved replacing the septa closure on the aqueous sample line with a Teflon® Mininert valve (Vici Precision Sampling, Baton Rouge, LA). This was installed to allow introduction of air after each sampling event and to eliminate the septa closures that tended to plug the syringe needle. The configuration for experiments completed during Trial 4 is shown in Figure 4-4.

The 120 mL glass vessel was filled 90 mL of the same TCE-DI stock solution used during the three previous trials, the vessel was sealed, and submerged in the 5 L water bath. After a 1-day equilibrium period, the water bath temperature was set to 30 °C and gas samples were collected after 2 hours. The gas sample was collected by pulling 2 mL of gas through the 250 μL sample loop that was heated to 120 °C while the aqueous line was open. This allowed air to flow into the vessel through the TCE-DI water before refilling the gas volume. After collecting three gas samples over a 30 min period, three aqueous samples were collected, and then the aqueous line was opened to allow air to flow into the vessel interior. The aqueous line was then closed and the temperature increased to 40 °C. This process of providing gas refill after each sample collection even was repeated at each temperature and the resulting Henry's Law coefficients (Figure 4-5) were on average of 6, 25, and 59% greater than had been determined during Trials 1, 2, and 3, respectively. The Henry's Law value at from Trial 4 at 30 °C was 0.95 ± 0.09, which was 88% greater than the 0.50 value reported by Staudinger and Roberts (2001).
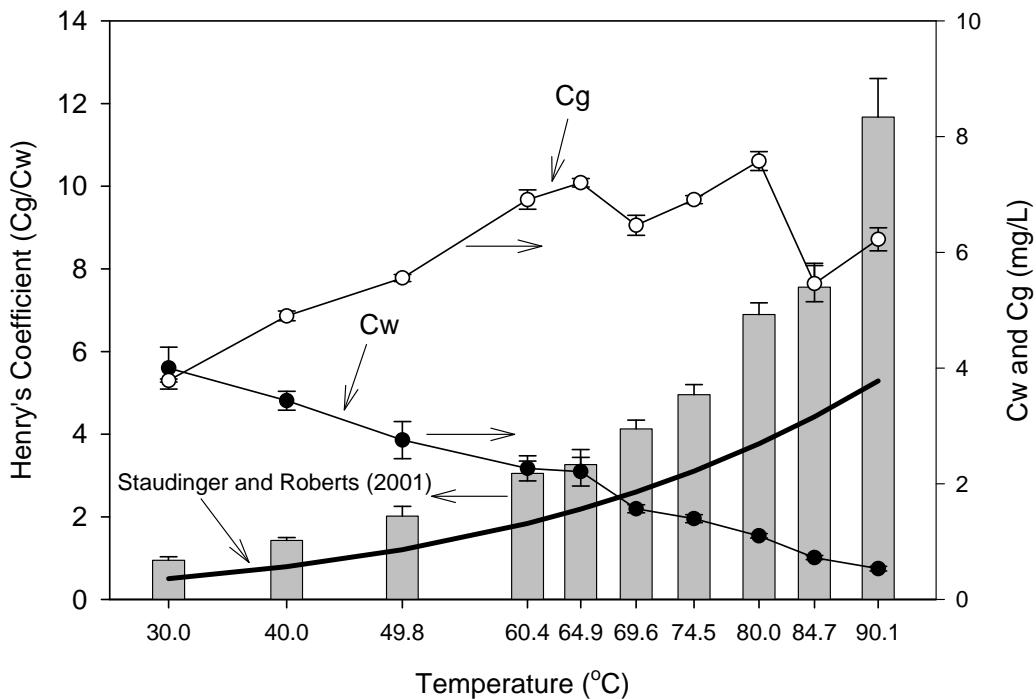
Figure 4-4. Results from Henry's Law experimental Trial 4 for TCE.

Although there was variability between the Henry's Law coefficients determined from each of the four experimental trials, there was a consistent increase in the values with increasing. Staudinger and Roberts (2001) suggested that the Henry's Law coefficient temperature dependence could be described by the following equation:

$$\log(\mathrm{H}) = a - \frac{b}{T} \tag{4.1}$$

where *a* and *b* are fitting coefficients, *H* is the dimensionless Henry's Law coefficient, and *T* is the temperature in Kelvin. Figure 4-5 shows a plot of the Henry's Law coefficient values from the four experimental trials along with Equation 4.1, for which the fitting coefficients were determined from a linear regression analysis of the data from Trials 1 through 4. The Henry's Law coefficient values from each trial fit the Log normal correlation (Equation 4.1) with an overall linear regression coefficient of 0.933, which supports the use of Equation 4.1 to describe the temperature dependence of Henry's Law coefficient between the temperatures of 24 and 90 °C. These results extend the upper temperature range of Equation 4.1 from 50 to 90°C.
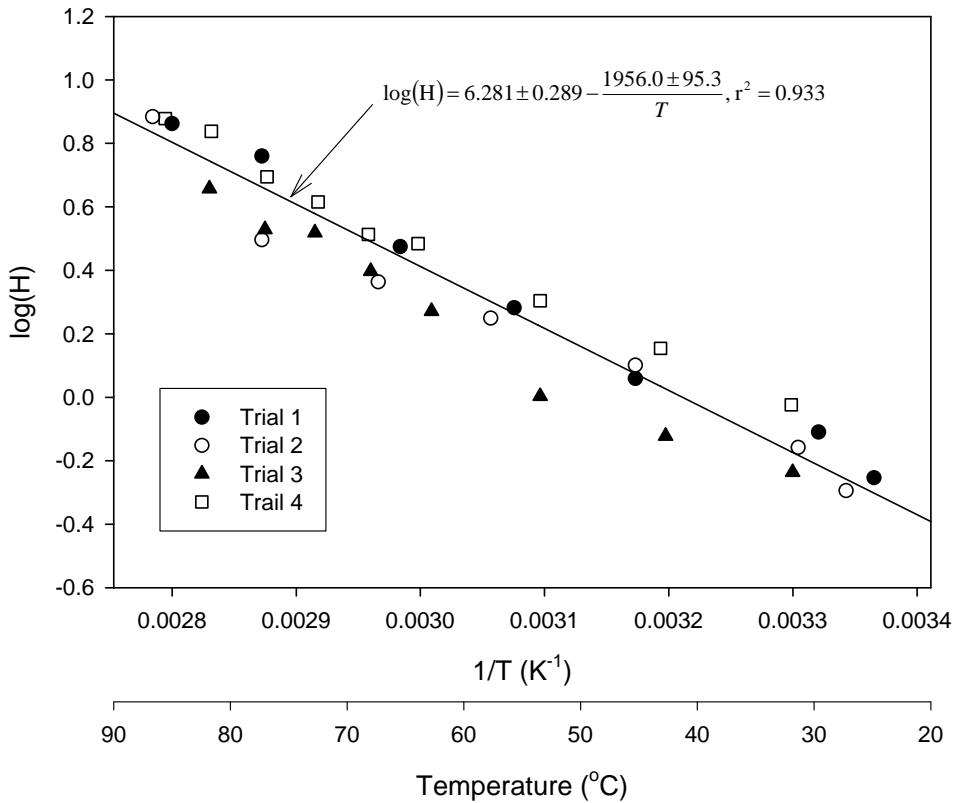
Figure 4-5. Correlation of results from Henry's Law experimental Trials 1, 2, 3 and 4.

Although Equation 4.1 provides a reasonable fit of the experimental data, use of additional temperature-dependent parameters provides a better fit and allows thermodynamic parameters such as the heat of vaporization, entropy, and heat capacity to be determined (Benson and Krause, 1976). The data from experimental Trials 1 through 4 were fit to an equation with the following form:

$$\ln(H_{yx}) = a_0 + a_1 \frac{1}{T} + a_2 \frac{1}{T^2} \tag{4.2}$$

where H_{yx} is the Henry's coefficient expressed as the mole fraction of TCE in the gas phase (y) divided by the TCE mole fraction of the aqueous phase (x), T is the absolute temperature in Kelvin, and a_0, a_1, and a_2 are fitting coefficients. The result of using Equation 4.2 to fit the data from experimental Trials 1 through 4 improved the regression coefficient from 0.933 to 0.949, and allowed the calculation of the thermodynamic parameters shown in Figure 4-7.
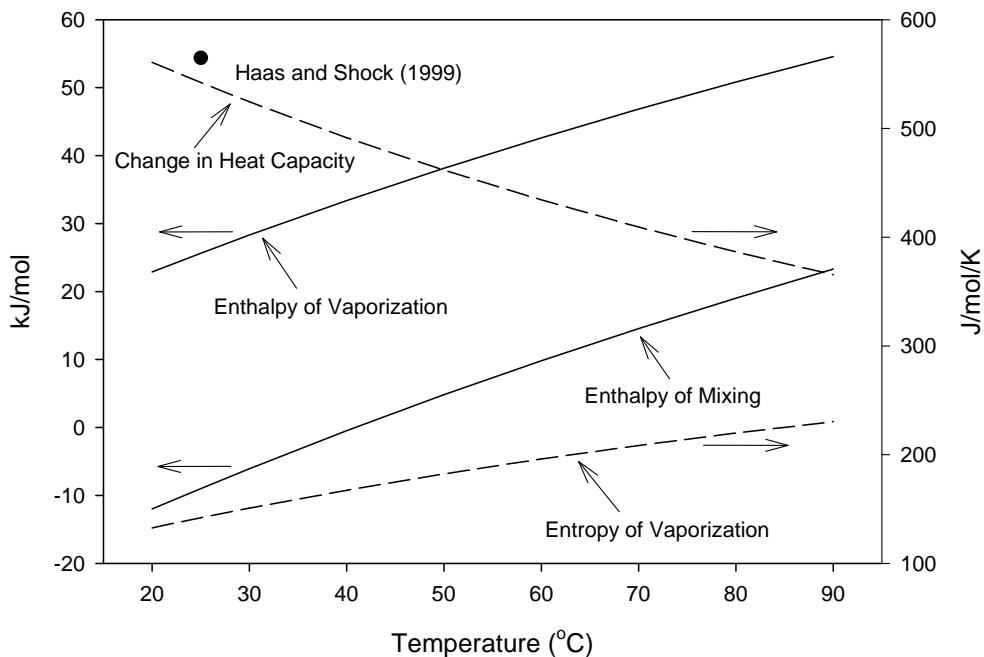
Figure 4-6. Thermodynamic values with increasing temperature for theoretical transfer of 1 mol of TCE from the dissolved phase to the gas phase calculated from experimental data derived parameters.

There are several insights into the energy required to transfer 1 mol of TCE from the dissolved phase to the gas phase that can be inferred from Figure 4-6. The most important behaviors are the increase with temperature of (a) the enthalpy of vaporization, the difference between the enthalpy of TCE in the gas phase and in the dissolved phase, and (b) the enthalpy of mixing, the difference between the enthalpy of TCE dissolved in water and neat TCE (note: neat TCE was not present in these experiments). This means that as the system temperature was increased, the solubility of TCE decreased and the thermodynamic driving force towards TCE condensing into a neat phase increased. The decrease in solubility is opposite to that behavior described by previous researchers who reported an increase in TCE solubility with temperature (e.g., Knauss et al., 2000; Heron et al., 1998a). The increase in enthalpy of mixing is consistent with the findings of Heron et al. (1998c) and is also consistent with the observation that pools of neat phase solvent can form during thermal remediation (i.e., solvent banks). The change in heat capacity shown in Figure 4-6 is the difference between the heat capacity value for TCE in the gas phase and TCE dissolved in water. The decrease in values for the change in heat capacity with temperature was expected based on previous work. The heat capacity value of 542.1 (J/mol/K) obtained from the results of Trials 1 through 4 at 25 ^{o}C was close to the value of 564.8 (J/mol/K) reported by Haas and Shock (1999) that was derived using the Helgeson-Kirkham-Flowers (HKF) equation of state. A final feature of Figure 4-6 concerns the entropy of vaporization, which increased with temperature. The increase in entropy was expected given that increasing temperature has a greater effect on the gas phase entropy than on the aqueous phase entropy. Overall, the thermodynamic trends shown in Figure 4-6 are consistent with the observed increase

in Henry's Law coefficients, where the most important finding was that TCE solubility is expected to decrease with increasing temperature.

The aqueous phase in the equilibrium system for Trials 1 through 4 was stirred with a magnetic stir bar. However, no magnetic stir bars are present in the subsurface during thermal treatment. Thus, a final experimental trial (Trial 5) was performed without a stir bar to determine the time to reach equilibrium. Trial 5 involved heating the equilibrium system to 70 °C and measuring the aqueous and gas phase TCE concentrations as a function of time. After 30.5 h at 70 °C, the value of Henry's Law coefficient was 1.4 (Figure 4-7), 60% less than the average value determined from the previous four trials where the aqueous phase was mixed. Therefore, the rate of TCE transfer from the aqueous to gas phase was limited without mixing. This result suggests that using Henry's Law coefficients determined from experiments that involved mixing may not be applicable for describing the partitioning of TCE in subsurface environments during thermal treatment where mixing is not anticipated to occur.
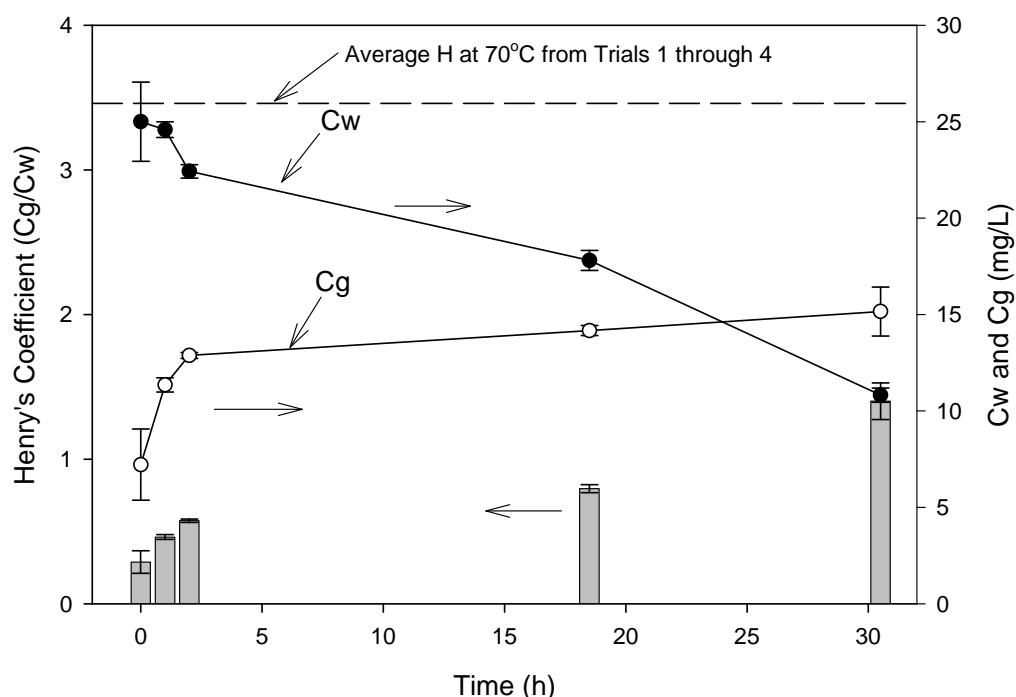
Figure 4-7. Results from Henry's Law experimental Trial 5 for TCE. Figure shows change in aqueous (C_w) and gas (C_g) concentrations of TCE with time for the equilibrium system at 70 °C. Note: the aqueous phase was static, not mixed.

Limiting transport of TCE to diffusion alone (i.e., no mixing) will increase the time required to achieve equilibrium conditions. A theoretical analysis provided by Crank (1975, p.38) considers the approach to equilibrium for a solute (e.g., TCE) between two phases or media with differing diffusion coefficients (e.g., water and gas), where the solute is initially present in only one phase (e.g., water). A period of 347 h would be required for the concentration of TCE in the aqueous phase at a location 5 cm below the water/gas interface to reach steady-state values based on an aqueous diffusion coefficient of 1×10^{-5} cm^2/s. By contrast, the gas phase diffusion coefficient is four orders-of-magnitude greater than for water, and the time to reach steady-state

concentrations is on the order of minutes. In past Henry's Law coefficient determinations, researchers reported that gas phase concentrations reached steady-state within 15 to 30 min (e.g., Shimitori and Arnold, 2002; Vane and Giroux, 2000). This was taken to indicate that aqueous concentrations were also at steady-state even though no aqueous samples were collected. The results of Trial 5 show that the aqueous phase concentrations continue to decline over 30 h, while the gas phase concentrations are relatively stable after 2 h. Thus, previous determinations of Henry's coefficients that relied only on gas phase concentration measurements to determine steady-state conditions are unlikely to represent equilibrium conditions.

A separate experimental series was performed according to the Equilibrium Partitioning in Closed Systems (EPICS) method (Gossett, 1987) as modified for use with automated headspace samplers by Shimitori and Arnold (2002) and Chai and Zhu (1998). At 70 °C, the Henry's Law coefficients we obtained for TCE ranged from 0.54 ± 0.12 to 1.42 ± 0.49, while Shimitori and Arnold (2002) reported a value of 2.01 ± 0.34. The two limitations to the EPICS approach are that 1) the system is assumed to be at equilibrium and 2) that there is no loss of chlorinated solvent from the headspace enclosure. To evaluate if TCE was at equilibrium, a headspace vial was loaded with only 10 μL of a 1,000 mg/L TCE methanol solution and the content of the vial was repeatedly determined using a headspace autosampler operated at 50 °C. Results of this analysis show that the TCE of the headspace vial linearly decreases with subsequent sampling events (Figure 4-8). Next, 10 μL of a 1,000 mg/L TCE methanol solution was added to a headspace vial containing 10 mL of deionized water. The vial with TCE and water was analyzed in the identical manner as the TCE only vial. The TCE content from the vial with deionized water increased after the second sampling event (45 min net incubation time, Figure 4-8) and then decreased with subsequent sampling events. The non-linear behavior of TCE in the vial with deionized water compared the linear result in the TCE only vial indicates that the TCE distribution was not at equilibrium in the TCE-deionized water vial (Kolb and Ettre, 2006). This result counters the claim by Shimitori and Arnold (2002) and Vane and Giroux (2000) that equilibrium within their headspace vials was achieved after 30 min of incubation.

Longer incubation times would theoretically allow TCE to reach an equilibrium distribution, however, our results obtained after incubating for up to 4 h in duration did not improve the Henry's Law values (data not shown). Longer incubation times tend to compromise the second EPICS assumption that no mass is lost from the vials during incubation. The vials are sealed with polymer septa, and TCE and PCE have been shown to be soluble in these septa and can readily diffuse through the septa at temperatures of 50 °C (Costanza and Pennell, 2007a). Thus, while EPICS is a reasonable method for determining Henry's law values at ambient temperatures, at higher temperatures the loss of solvent due to diffusion through the septa violates the assumption of mass conservation and renders this method inappropriate.
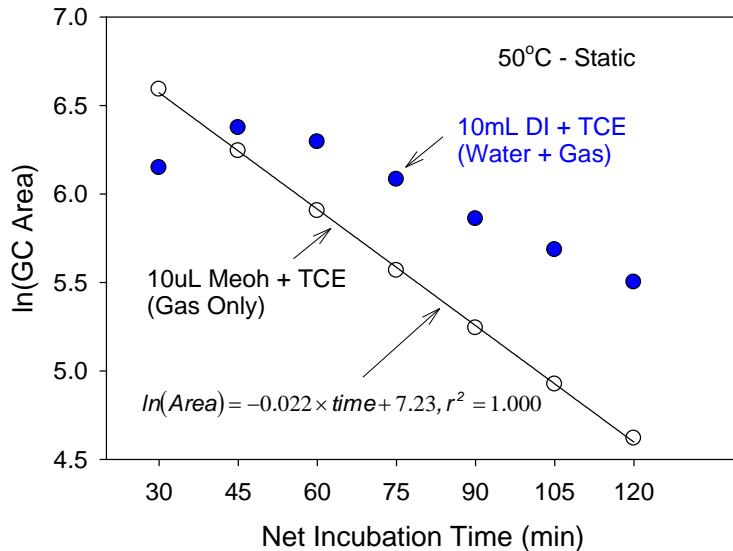
Figure 4-8. Amount of TCE in headspace vials with only gas phase TCE, and with TCE and DI water at 50 °C.

4.1.2. Aqueous Solubility of TCE as a Function of Temperature

Experiments were completed to measure the solubility of TCE with increase in temperature using the same equilibrium system used to determine the Henry's Law coefficients (described in Section 3.2). Results from this experiment were that TCE solubility was relatively constant at 1139±99 mg/L between 25 and 85°C (Figure 4-9). This result is in contrast to the data of Knauss et al. (2000) and Heron et al. (1998), who reported that TCE solubility increased by 1.3 and 1.2 times, respectively, by increasing the temperature from 25 to 70°C. The results reported by Knauss et al. (2000) were obtained for a liquid-phase system, that was equilibrated from 7 to 14 days between each isothermal temperature under a confining pressure of at least 10 bar. Heron et al. (1998) measured the TCE content in the effluent from a TCE saturated, 20 cm long by 2 cm diameter, sand-filled column (i.e., column generator) after equilibrating at each isothermal temperature for 2 hours. Both of these techniques could have resulted in super-saturated conditions that are not expected to occur during thermal remediation, where Heron et al. (1998) reported that the aqueous samples from their TCE-saturated column appeared cloudy at higher temperatures. The solubility values shown in Figure 4-9 were obtained using the equilibrium vessel containing TCE-NAPL and water which was not agitated during these experiments and the aqueous phase remained clear over all temperatures.
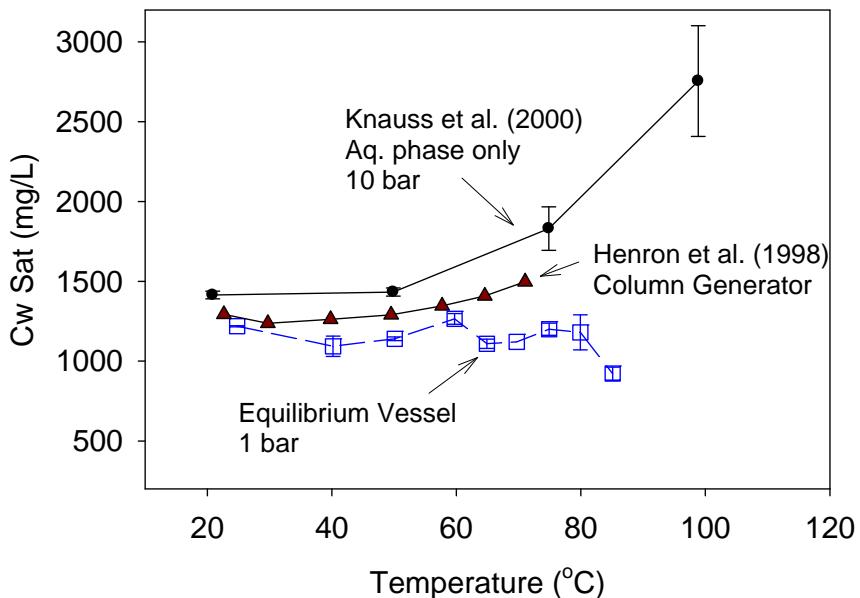
Figure 4-9. Measured aqueous solubility of TCE with increasing temperature.

Another way to view the solubility data shown in Figure 4-9 is to demonstrate its impact on Henry's Law coefficients. This can be accomplished by calculating Henry's Law coefficient using the following formula:

$$H = \frac{P^0_{TCE}}{C_{w,sat}} \tag{4.3}$$

where P^0_{TCE} is the vapor pressure of TCE-NAPL and $C_w Sat$ is the aqueous solubility of TCE. Henry's Law coefficients were calculated using Equation 4.3 with vapor pressure of neat TCE obtained using the correlation reported by Linstrom and Mallard (2005) and the TCE solubility values shown in Figure 4-9. The calculated Henry's Law coefficients based on Equation 4.3 are provided in Figure 4-10 along with the Henry's Law correlation developed from measurements completed with dilute TCE-water solutions (i.e., Equilibrium System Correlation) as shown in Figure 4-5.
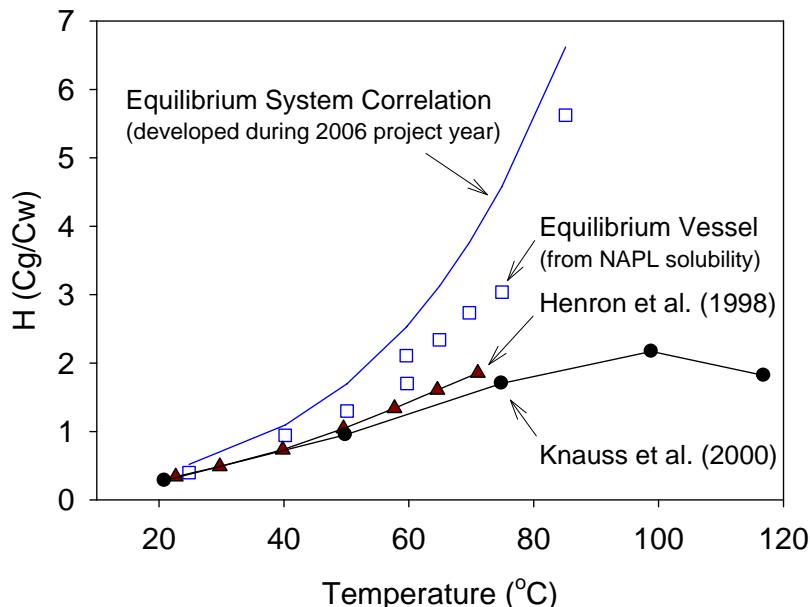
Figure 4-10. Henry's Law coefficients calculated from measured TCE aqueous solubility and TCE-NAPL vapor pressure values. Also shown is the correlation provided in Figure 4-5.

The Henry's Law coefficients calculated using Equation 4.3 were within 23% of those predicted from independent measurements of dilute TCE-water solutions (Figure 4-11). While the TCE solubility values determined during this project predicts that Henry's Law coefficients will significantly increase with temperature (e.g., 12 times increase from 25 to 85°C), the solubility values determine by Knauss et al. (2000) and Heron et al. (1998) suggest more modest gains in Henry's Law coefficients with temperature. The solubility measurements were obtained using three different methods where the results reported herein were from a static batch system at atmospheric pressure (i.e., 1 bar). Knauss et al. (2000) also used a batch system; however, only water was present in their system and consequently pressures of 10 bar were used to prevent formation of a gas phase with increase in temperature. Heron et al. (1998) used a generator column with stationary TCE-NAPL and water flowing through the column. All three methods have disadvantages where the effect of pressure may have led to supersaturated conditions (Knauss et al., 2000), flowing water may have dislodged stationary TCE-NAPL (Henron et al., 1998), and the system employed for this work may not have been at equilibrium due to lack of mixing. The method chosen for this project was thought to simulate subsurface conditions anticipated to occur during thermal treatment, with the exception of soil being present. Thus, we claim that aqueous TCE concentrations will remain relatively constant with increase in subsurface temperature.

4.1.3. Desorption of Chlorinated Ethenes from Field Soils with Increasing Temperature

Equilibrium sorption isotherms as a function of temperature were measured as part of incubating soil collected from Camelot Cleaners, Fort Lewis, and Great Lakes sites. The soil-water distribution coefficient (K_d) was calculated based on the soil concentration (C_s) and the

aqueous concentration (C_w) of TCE or PCE at each incubation temperature and time using the following equation:

$$K_d = \frac{C_s}{C_w} \qquad\qquad (4.4)$$

The soil concentration was determined by adding 15 mL of methanol to each ampule after draining the free water, followed by transfer of the soil-methanol slurry to a screw-top vial that was allowed to equilibrate for at least 24 h prior to analyzing the methanol for TCE and/or PCE content. Aqueous-phase concentrations were determined from 1 mL water samples collected immediately after opening each ampule.
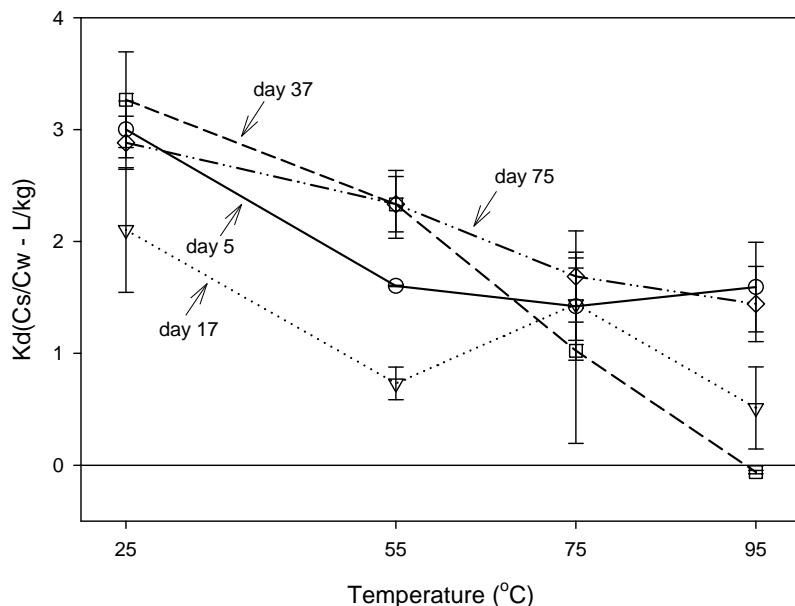
Figure 4-11. Sorption coefficients determined for PCE desorption from Camelot soil.

The soil-water sorption coefficients for PCE desorbing from the Camelot soil tended to decrease with temperature and were less dependent on incubation time (Figure 4-11). The average K_d values for the Camelot soil determined for ampules incubated over 75 days were 2.7 ± 0.2 (L/kg) at 25 ^{o}C, 1.3 ± 0.2 at 55 ^{o}C, 1.1 ± 0.7 at 75 ^{o}C, and 0.9 ± 0.8 at 95 ^{o}C. These results indicate that heating the ampules to temperatures greater than 55 ^{o}C caused PCE mass to transfer from the solid phase to the aqueous phase. This observation supports the suggestion that raising subsurface temperature can increase the rate of chlorinated solvent recovery by increasing concentrations in the extractable aqueous and gas phases (Davis, 1997).

The soil-water sorption coefficient obtained for TCE amended to the Fort Lewis soil also decreased with temperature (Figure 4-12). However, these decreases were not as great as those observed for PCE in the Camelot soil. The average K_d values for TCE added to Fort Lewis soil determined for ampules incubated over 95.5 days were 0.5 ± 0.1 at 25 ^{o}C, 0.5 ± 0.1 at 50 ^{o}C, and

0.3 ± 0.1 at 95 °C. Therefore, heating Fort Lewis soil had less effect on the transfer of TCE mass than was observed for PCE in the Camelot soil.
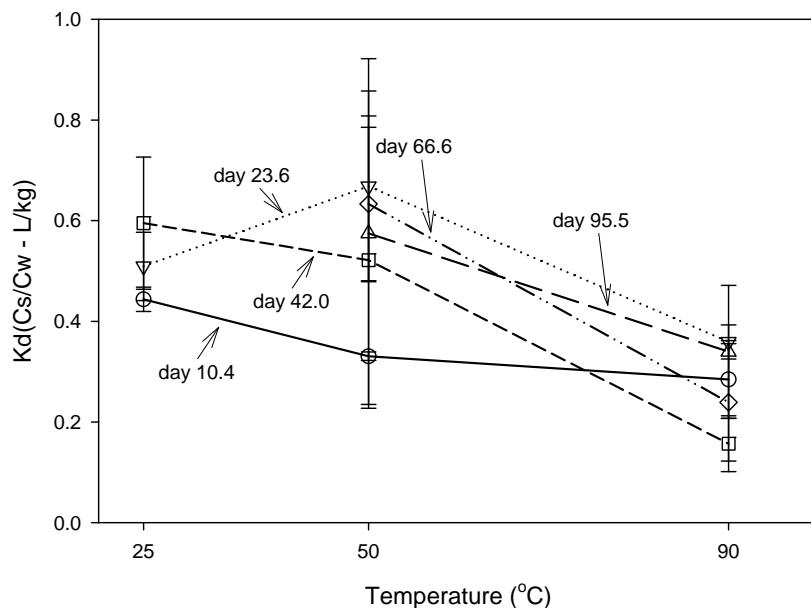
Figure 4-12. Sorption coefficients determined for TCE with Fort Lewis soil.

A large decrease in soil-water sorption coefficient was observed for PCE desorption from Great Lakes soil (Figure 4-13). The average K_d values for Great Lakes soil, determined for ampules incubated over 184.9 days, were 8.7 ± 1.0 at 25 °C, 3.0 ± 0.6 at 50 °C, 2.9 ± 0.4 at 70 °C and 1.8 ± 0.5 at 95 °C. The observed decrease in the soil-water distribution coefficient was due to the increase in aqueous phase concentration, whereas the soil concentration remained constant at 376 ± 17 mg/kg over 37.5 days. To determine the total mass of PCE contained within the Great Lakes soil, a series of methanol extractions was performed with soil that had been incubated for 37.5 days. The concentration of PCE in the 15 mL of methanol added to the soil from each ampule was determined after three days of equilibration time; the methanol was drained, and 15 mL of fresh methanol added. As shown in Figure 4-14, the concentration of PCE in the Great Lakes soil decreased with each subsequent extraction, and PCE was still detected after five methanol extractions. After the initial extract, the total concentration of PCE was 291 ± 18 mg/kg and increased to 641 ± 54 mg/kg after five methanol extractions. Of the total amount of PCE detected in the ampule phases, 54.6% remained strongly sorbed to the Great Lakes soil even after heating for 185 days at 95 °C.
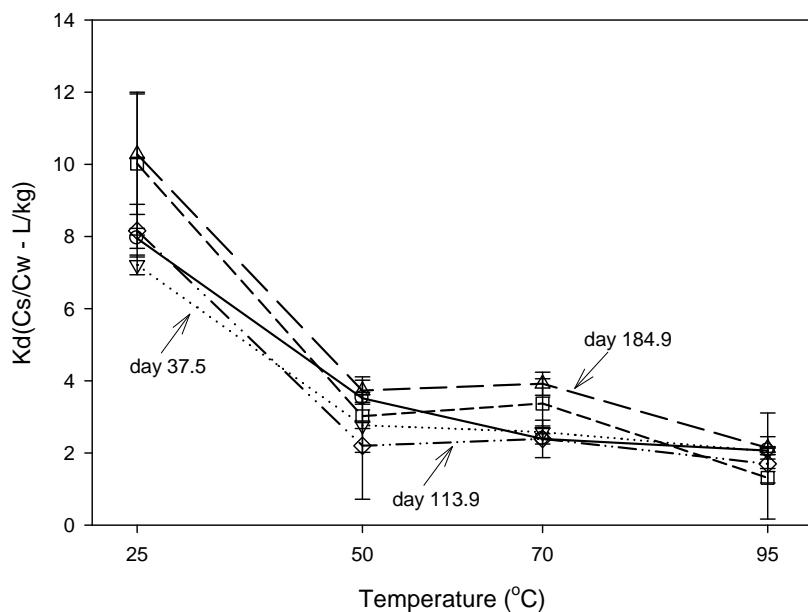
Figure 4-13. Sorption coefficients determined for PCE desorption from Great Lakes soil.
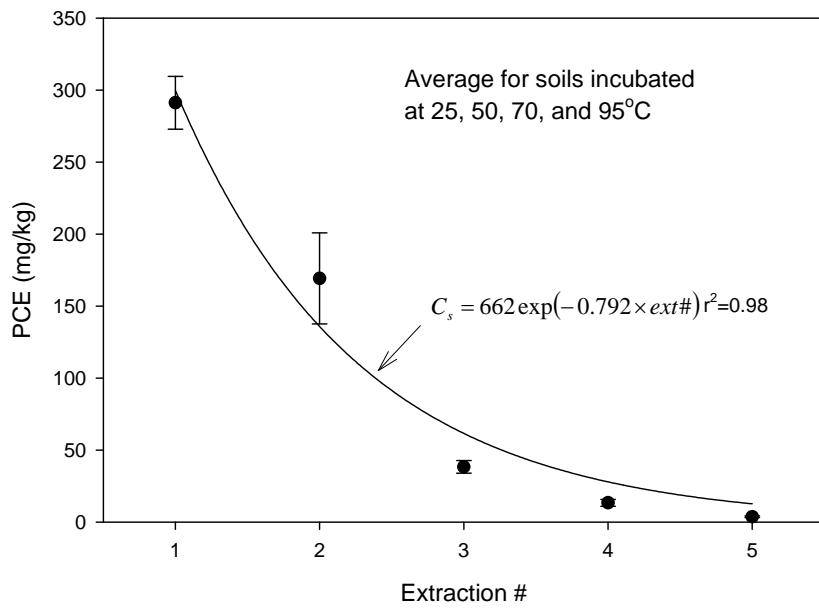
Figure 4-14. Great Lakes soil PCE concentration after each sequential methanol extraction.

4.2. CHEMICAL REACTIVITY AND BYPRODUCT FORMATION

4.2.1. Reactivity of PCE in Water as a Function of Temperature

Experiments were completed with PCE in oxic and anoxic deionized water (no solids present) at initial concentrations of 10 mg/L, similar to the experiments previous completed with TCE (Costanza et al., 2005). Each experiment involved 80 ampules, with 20 ampules incubated at each temperature of 25, 55, 75, and 95 °C. The range of temperatures was chosen to determine the Arrhenius pre-exponential and activation energy parameters required to predict the rate of PCE decay at other temperatures. The ampule contents were analyzed for oxidation products including CO, CO_2, chloride, and organic acids, along with reduced products including TCE, *cis*-DCE, and VC. Figure 4-15 shows the amounts of PCE in anoxic ampules incubated at 25 and 95 °C. The amount of PCE in each ampule was calculated based on the aqueous and gas phase concentrations estimated using Henry's Law ($C_g = HC_w$), with the dimensionless Henry's Law constant (H) equal to 0.699. This approach was used to correct for the slight differences in the volume of water initially added to each ampule. Also shown in Figure 4-15 are the amounts of chloride and CO_2 detected in ampules incubated at 95 °C.
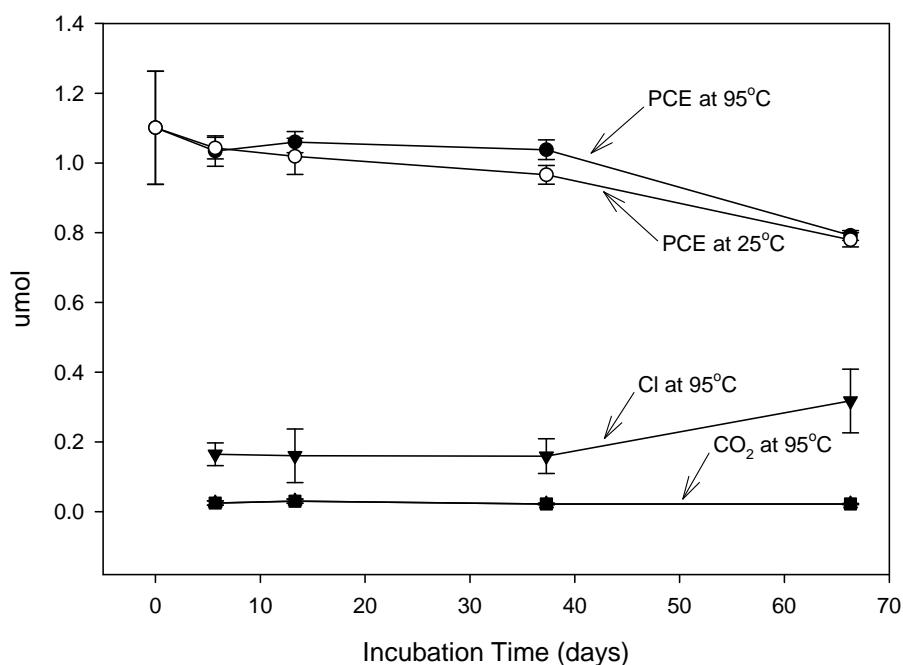
Figure 4-15. Amounts of PCE, chloride (Cl) and carbon dioxide (CO_2) in ampules that contained anoxic deionized water incubated at 95°C.

Slight decreases in the amounts of PCE with incubation time were observed in the anoxic ampules, however, the was no statistical difference ($p > 0.05$) between the amount of PCE in the ampules maintained at 25 °C and that in ampules incubated at 95 °C with the exception of the 37 day result, where the amount of PCE in ampules incubated at 95 °C was greater than ($p < 0.05$) the amount in ampules stored at 25 °C. The chloride levels were similar over the initial 37 days of incubation at 95 °C in the anoxic ampules (Figure 4-15), but increased after 70 days of incubation. This increase in chloride concentration may represent PCE degradation. However,

additional reaction products, including CO_2, CO, and formate, that were expected based on the results for TCE experiments were either not detected (CO and formate), or were detected at concentrations near the analytical detection limit for CO_2. Although there was a decrease in PCE levels during the incubation, no corresponding increase in expected degradation products was observed. Thus, abiotic PCE degradation was not considered to have occurred in anoxic ampules incubated at 95 °C for 70 days.
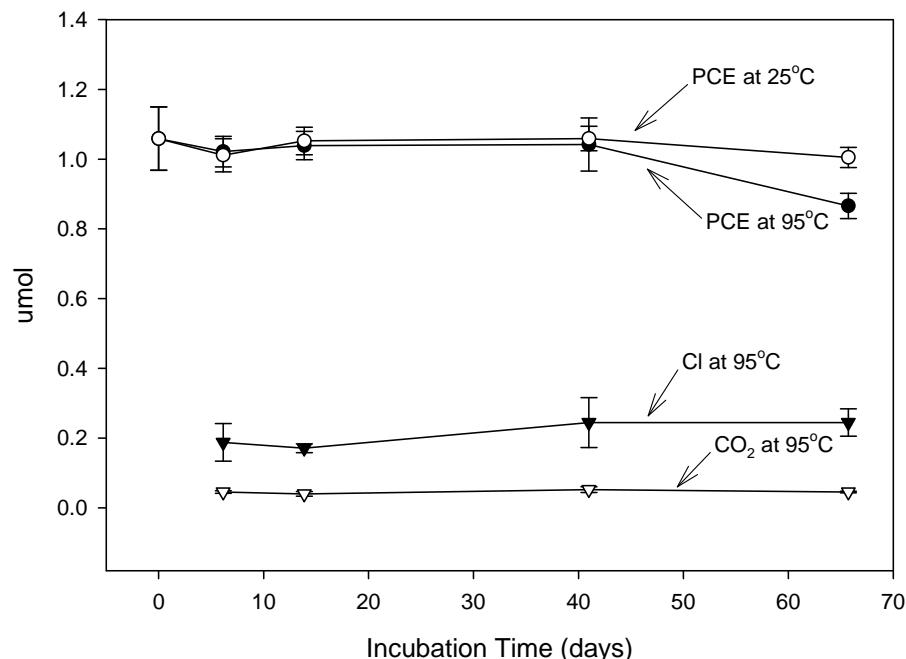
Figure 4-16. Amounts of PCE, chloride (Cl) and carbon dioxide (CO_2) in ampules that contained oxic deionized water incubated at 95°C.

Figure 4-16 shows the amounts of PCE, chloride, and CO_2 in oxic ampules containing ambient levels of oxygen (dissolved oxygen = 8 mg/L and gas phase = 21% O_2). The PCE levels in the oxic ampules decreased slightly over a 75 day period at 95°C and the levels of chloride and CO_2 in the oxic ampule incubated at 95°C were similar between each incubation period. Hence, as with the anoxic ampules, PCE was not considered to have degraded in the oxic ampules after 75 days at 95°C.

4.2.2. Evaluation of VOA Vials for PCE and TCE Thermal Reactivity Experiments

Recently, Brown et al. (2005) and Brown (2006) reported results from a single-phase aqueous system that was used to determine the rate of PCE and TCE hydrolysis at 35, 45, and 55 °C. The half-life for the disappearance of TCE at 45 °C was 17 days based on data reported in Brown (2006), which is 129 times faster than the half-life at 45°C based on Arrhenius rate parameters reported by Knauss et al. (1999). The faster rate reported by Brown (2006) was obtained using a system, which was completely filled with aqueous phase, consisting of glass volatile organic analysis (VOA) vials sealed with Teflon®-lined silicone rubber septa. The advantage of the VOA vials is that the flexible rubber septa can accommodate the expansion of liquid water which occurs with increasing temperature. This feature allows the vials to be

completely filled with water at room temperature and then incubated at elevated temperatures without the glass enclosure breaking due to liquid water expansion. Knauss et al. (1999) overcame this problem by using a custom made apparatus consisting of a variable volume, gold-walled vessel that was contained within a water-filled steel enclosure pressurized to 1,000 kPa. Thus, the VOA vials appear to offer a relatively low-cost and readily available alternative to the custom made gold-walled system employed by Knauss et al. (1999) for the study of aqueous-phase dechlorination reactions in a zero headspace enclosure at elevated temperatures. However, Brown (2006) only analyzed the VOA vial contents for the parent compound, either PCE or TCE, and did not demonstrated that these compounds were being dechlorinated by analyzing for hydrolysis products such as chloride.

Volatile organic analysis vials are the recommended container for transporting aqueous samples containing dissolved-phase volatile organic compounds from the field to the laboratory (ASTM, 2004). However, the samples must be stored at 4 °C and analyzed within 14 days of collection to avoid loss of volatile organic compounds, such as PCE and TCE. Even with these precautions, PCE and TCE have been shown to be sorbed by Teflon®-lined septa, leading to losses in contaminant mass from the aqueous phase (Kovacs and Kampbell, 1999). The integrity of the seal between the Teflon®-lined septa and the vial rim also suffers from imperfections in the glass surface that can lead to additional losses of PCE and TCE from VOA vials (Schumacher et al., 2000). Although VOA vials are routinely used for low temperature storage of volatile organic compounds, there are no reports regarding their adequacy for use as enclosures at elevated temperatures.

Therefore, the objective of this study was to determine if 1) VOA vials were suitable for the study of aqueous-phase dechlorination reactions at elevated temperatures and 2) using single-phase containing VOA vials resulted in faster dechlorination rates compared with the rates obtained from two-phase containing flame-sealed ampules.

4.2.2.1. Changes in PCE and TCE Concentrations

The concentration of PCE in the VOA vials incubated at 21 and 55 °C decreased with time (Figure 4-17), based on analytical results for a 1 mL aqueous sample collected from each vial. The rate of PCE concentration decrease was greatest in the VOA vials maintained at 55 °C and followed a first-order decay model with a half-life of 11.2 days. Brown (2006) reported data for the disappearance of PCE with time at 45 °C from which a half-life of 12 days was calculated, and this value was within 6 % of the 11.2 day half-life determined herein at 55 °C. The rate of PCE concentration decrease at 21 °C also followed a first-order decay model with a half-life of 77.6 days. While PCE was disappearing from the VOA vials, there was no increase in chloride or relevant transformation products (e.g., cis-1,2-dichloroethylene) to support the conclusion that PCE was being dechlorinated. The concentration of TCE in VOA vials also decreased with incubation time (Figure 4-18), following a first-order decay model with a half-life of 262 days at 21 °C and 21.1 days at 55 °C. The half-life based on the data presented in Brown (2006) for TCE at 45 °C was 17 days which was within 22 % of the 21.1 day half-life determined herein at 55 °C. The concentration of chloride in the VOA vials with TCE was also consistent at 0.05 ± 0.02 mg L^{-1} for the 36 vials incubated at 21 and 55 °C over the 37.8 day period. Organic acids including formate, acetate, glycolate, and oxalate were not detected in aqueous samples above the 10 μM detection limit.
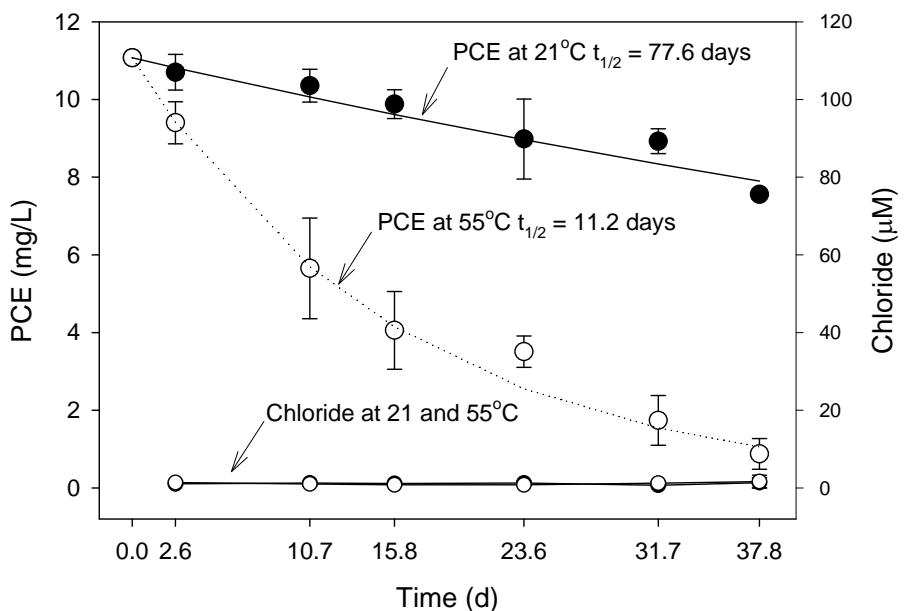
Figure 4-17. The concentration of PCE and chloride in VOA vials incubated at 21 and 55 ^{o}C over a period of 37.8 days. Each data point represents the average of three VOA vials and error bars correspond to one standard deviation.
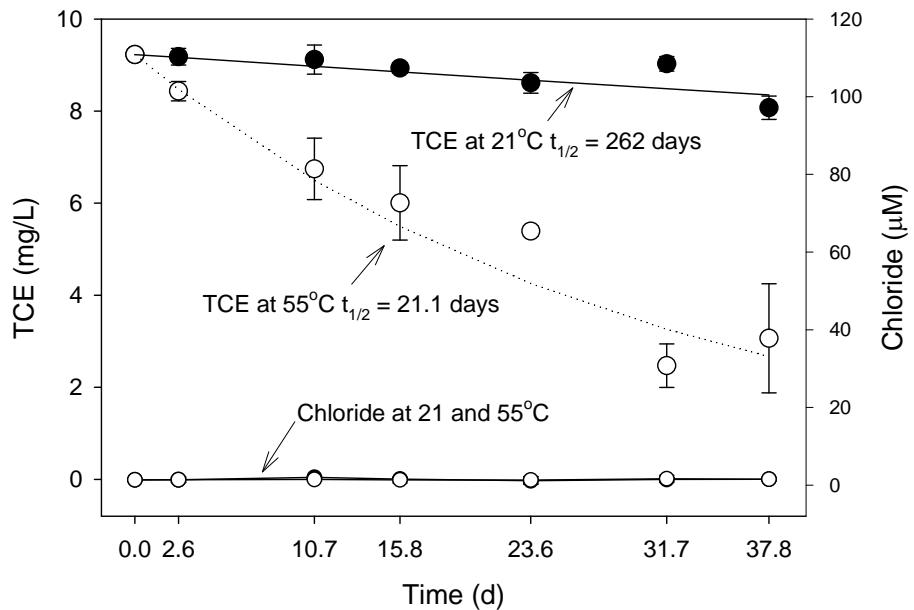
Figure 4-18. The concentration of TCE and chloride in VOA vials incubated at 21 and 55 ^{o}C over a period of 37.8 days. Each data point represents the average of three VOA vials and error bars correspond to one standard deviation.

Brown et al. (2005) and Brown (2006) also observed decreasing PCE and TCE concentrations in VOA vials incubated at 55 °C and suggested that the disappearance was due to a thermally-induced dechlorination reaction. If PCE and TCE were being dechlorinated, then an increase in chloride concentration would be expected after transforming into molecules with fewer chlorine atoms (e.g., *cis*-DCE). However, rather than increasing, the concentration of chloride in the VOA vials containing PCE was consistent at 0.04 ± 0.02 mg/L for the 36 vials incubated at 21 and 55 °C over the 37.8 day period. Thus, the observed reductions in aqueous phase concentrations of PCE and TCE in the VOA vials was attributed to PCE and TCE loss from the vials, rather than a thermally-induced dechlorination reaction. The potential pathways by which volatile compounds can escape from VOA vials include volatile losses due to poor seal integrity, sorption by the Teflon®-lined silicone septa, and diffusion through the septa.

4.2.2.2. Integrity of VOA Vial Seals

Seal integrity refers to the seal between the Teflon®-lined silicone septa and glass lip of the VOA vial, which may be compromised by imperfections in the glass lip and septa surfaces (Schumacher et al., 2000). The seal integrity was evaluated by recording the initial mass of each vial prior to incubation and the final mass prior to sampling; a decrease in VOA vial mass after incubation potentially indicating poor seal integrity. The vial mass uniformly decreased for both the PCE- and TCE-containing VOA vials incubated at 21 °C decreasing by 0.31 ± 0.08 g or less than 0.5% of the total vial mass, while the 55 °C vials decreased by 0.68 ± 0.10 g or 1.0% of the total vial mass. Although there was no correlation between incubation time and mass decrease, there was a significant difference between the mass decrease of the 21 and 55 °C ampules ($p < 0.02$). This result suggests that more mass was lost from the 55°C ampules than from the vials incubated at 21 °C, potentially through the septa to glass seal. However, the uniformity of the mass loss and lack of correlation with incubation time may also be attributed to differences in the amount of water residing between the polypropylene screw-cap and glass threads. Although there were decreases in VOA vial mass during the incubation period that potentially indicated loss of vial contents or poor seal integrity, the mass decrease could also represent loss of water from the space between the screw-cap and glass threads.

4.2.2.3. Sorption of PCE and TCE by VOA Vial Septa

Kovacs and Kampbell (1999) reported that the concentration of dissolved-phase PCE and TCE, after incubating for 14 days at 25 °C, decreased by 5% to greater than 20% with an increase in the surface area of Teflon® within the vials from 284 to 2,998 mm². Because the concentration decreases were correlated with increase in surface area, sorption to Teflon® could have contributed to the losses of PCE and TCE from the 40 mL VOA vials. The mass of PCE sorbed by the septa for the experiments reported herein was determined for the VOA vials that had been incubated for 31.7 days. Immediately after collecting the 1 mL aqueous sample, the septa from each vial was removed, wiped dry with a paper towel, cut into small pieces, and then sealed in a headspace vial. The headspace vials containing the VOA vial septa were analyzed by the same automated headspace procedure used to determine the concentration of PCE in aqueous samples. The concentration of PCE in the septa was determined to be 0.82 ± 0.16 mg/kg from the vials at 55 °C, and 1.00 ± 0.35 mg/kg from the vials at 21 °C demonstrating that PCE had entered into the septa. The amount of PCE contained in the septa represented less than 0.5% of the mass missing from the 55 °C VOA vials and less than 2% of the mass missing from the 21

°C VOA vials after 31.7 days. Similarly, the concentration of TCE in the septa was 0.77 ± 0.16 mg/kg from the vials at 55 °C and 0.44 ± 0.26 mg/kg from the vials at 21°C demonstrating that TCE was also sorbed by the septa. The amount of TCE in the septa was less than 0.5% of the mass missing from the 55 °C VOA vials, but ranged from 3% up to 66% of the mass missing from the 21 °C VOA vials after 31.7 days. Thus, sorption alone could not account for the mass of PCE or TCE missing from the VOA vials.

4.2.2.4. Diffusion of PCE and TCE through VOA Vial Septa

Brown (2006) concluded that the decrease in PCE and TCE concentration was not due to loss of these compounds from the VOA vials. This conclusion was based on the observation that the neat PCE vapor pressure at 55°C is 10.4 kPa (Linstrom and Mallard, 2005), which is less than the vapor pressure of 34.0 kPa for neat TCE at 55°C, while the rate of PCE loss from the vials was greater than for the loss of TCE. Transport of volatile compounds through polymer membranes is described by the solution-diffusion model where volatile compounds dissolve into the polymer and diffuse through the membrane due to the concentration gradient across the membrane (Baker, 2004). Although the rate of permeation through polymer membranes is proportional to the vapor pressure of each compound, the effective vapor pressure for dissolved-phase volatile compounds is proportional to the Henry's Law coefficient multiplied by the aqueous concentration rather than the neat vapor pressure cited by Brown (2006). The dimensionless Henry's Law coefficient for PCE at 55 °C is 2.73, and the value for TCE at 55 °C is 1.49 based on the correlations reported in Staudinger and Roberts (2001).

Thus, the vapor pressure of PCE from an aqueous solution would be greater than that of TCE assuming both solutions contained equal dissolved-phase concentrations. The solution-diffusion model predicts that PCE would be transported through the VOA vial membrane at a rate that is 1.84 times faster than for TCE based on the ratio of Henry's coefficients (Table 4.1). Assuming that the rate of PCE and TCE disappearance from the VOA vials measured in this study was due to transport through the septa, then the first-order rate coefficients reported in Figures 4.17 and 4.18 should correspond proportionally to the rate of transport through the membrane. The ratio of the rate coefficients for the disappearance of PCE to that of TCE at 55 °C was 1.89, which was within 4% of the value predicted by the ratio of Henry's coefficients. This analysis supports the hypothesis that PCE and TCE loss from the VOA vials at 55 °C was due to diffusion through the Teflon®-lined silicone septa.

Table 4-1. Henry's Law coefficients and first-order rate coefficients for the disappearance of PCE and TCE from VOA Vials at 55 °C.

	Henry's Coefficient (C_g/C_w) (Staudinger and Roberts, 2001)	First-Order Rate Coefficient (1/day)
PCE	2.73	-0.0622
TCE	1.49	-0.0328
PCE/TCE	1.84	1.89

At 21 °C, the ratio of Henry's coefficients for PCE and TCE is 1.70, whereas the ratio of first-order rate coefficients was 3.42, or two times the value predicted by the solution-diffusion model (Table 4.2). This result suggests that the disappearance of PCE and TCE from the 21 °C vials was driven by more than the vapor pressure estimated from Henry's Law. Transport of organics through polymer membranes also depends on the solubility of the organic compound in the polymer. Therefore, the increase in rate of PCE disappearance from the VOA vials at 21 °C compared to the rate of TCE loss suggests that PCE solubility in the Teflon®-lined septa was greater than that of TCE. This conclusion is supported by the finding that the rate of sorption of PCE to groundwater monitoring well casing made from Teflon® was 3.22 times faster than for TCE at 25 °C (Parker and Ranney, 1994).

Table 4-2. Henry's Law coefficients and first-order rate coefficients for the disappearance of PCE and TCE from VOA Vials at 21 °C.

	Henry's Coefficient (C_g/C_w) (Staudinger and Roberts, 2001)	First-Order Rate Coefficient (1/day)
PCE	0.56	-0.0089
TCE	0.33	-0.0026
PCE/TCE	1.70	3.42

4.2.2.5. Comparison to Ampule Experiments

As with the VOA vials, the concentration of PCE in the flame-sealed glass ampules incubated at 25 and 55 °C decreased with time (Figure 4-19), however, the rate of decrease was not as rapid. The concentration of PCE in the 55 °C ampules was significantly greater than (P < 0.05) that found in the 25 °C ampules with the exception of the 13.3 day results where there was no difference ($p > 0.05$). The rate of PCE concentration decrease was greatest in ampules maintained at 25 °C, and followed a first-order decay model with a half-life of 209 days. The PCE concentration decrease at 55 °C also followed a first-order model with a half-life of 386 days, which was 34 times slower than the loss of PCE from the VOA vials at 55 °C. The loss of PCE mass from the ampules was not thought to represent PCE dechlorination because the concentration of chloride in the ampules was consistent at 0.2 ± 0.3 mg L^{-1} in the 25 °C ampules and 0.3 ± 0.3 mg/L in the 55 °C ampules over the 66.3 day incubation period. Gas phase carbon oxidation products including CO and CO_2 were either below the detection limit, CO was not detected above the 16 ppmv detection limit, or were at low and constant levels, average CO_2 levels were at 88 ± 12 ppmv for the 25 °C ampules and 128 ± 44 for the 55 °C ampules. Organic acids were not detected in aqueous samples above the 10 μM detection limit.
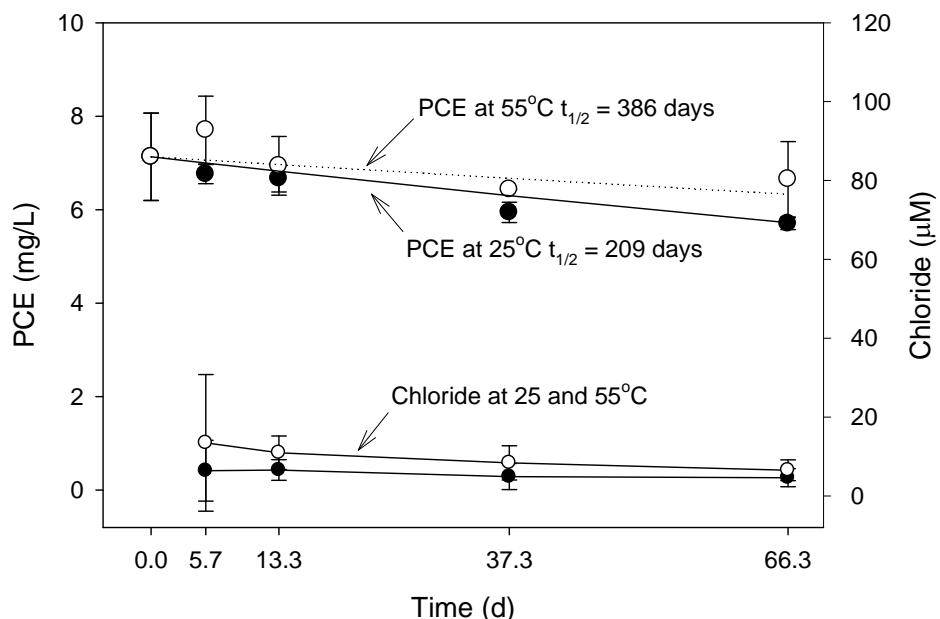
Figure 4-19. Concentration of PCE and chloride in ampules incubated at 25 and 55 °C over a period of 66.3 days. Each data point represents the average of five ampules and error bars correspond to one standard deviation.

The concentration of TCE in the flame-sealed ampules that contained groundwater amended with TCE increased with incubation time at 55 °C with the exception of a decrease after 95.5 days (Figure 4-20). The concentration of TCE in ampules maintained at 25 °C decreased over the initial 42 days, and then increased after 66.6 and 95.5 days. The chloride levels in the ampules with groundwater were greater than those observed in ampules and vials that were prepared with DI water; however, the chloride levels (2.0 ± 0.1 mg/L) were constant over the incubation period. Thus, there was no indication that TCE underwent a dechlorination reaction in ampules incubated at 55 °C over the 95.5 day period, and the slight increase in TCE concentration was attributed to reduced adsorption to the glass surface at 55 °C. Gas phase CO was not detected above the 16 ppmv detection limit in the 25 °C ampules, and was at a low and constant level in the 55 °C ampules at 50 ± 2 ppmv. Average CO_2 levels were at $8,681\pm1,091$ ppmv for the 25 °C ampules and $7,422\pm156$ for the 55°C ampules. Organic acids were not detected in aqueous samples above the 10 μM detection limit, while nitrate and sulfate were present at ca. 60 μM representing anions found in the groundwater that was used as the background solution for these ampules.
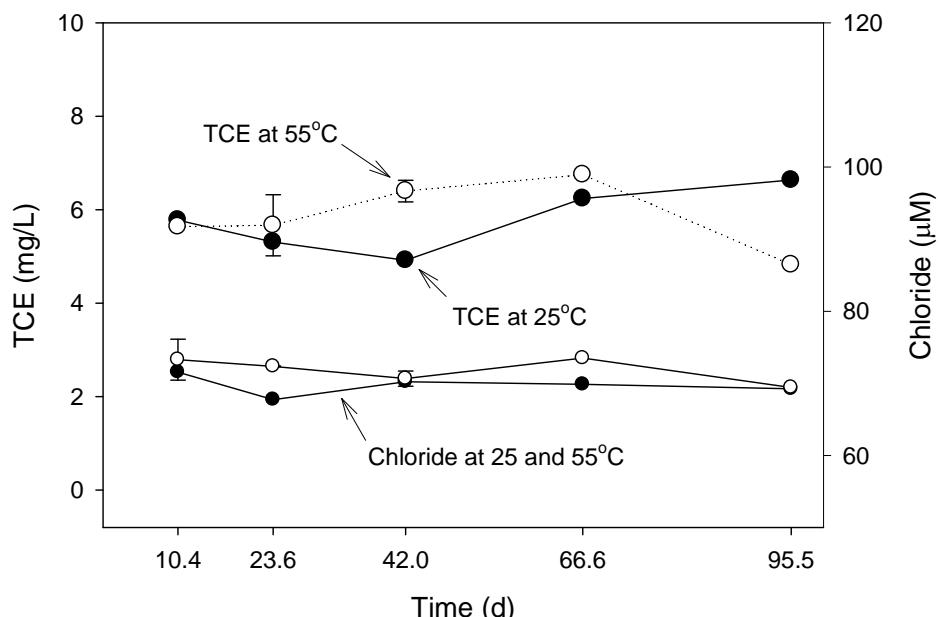
Figure 4-20. The concentration of TCE and chloride in ampules with groundwater from Fort Lewis, WA incubated at 25 and 55 °C over a period of 95.5 days. Data points through 42 days for the 55 °C ampules are the average of two ampules and error bars correspond to one standard deviation, while data points from 66.6 and 95.5 days are from one ampule. Data points for 25 °C are from a single ampule.

While decreases in PCE concentrations of up to 29% at 25 °C and 16% at 55 °C were observed in the flame-sealed glass ampules, the chloride levels were consistent over the 66.3 day incubation period. Thus, dechlorination of PCE was not considered to have occurred in ampules incubated at 25 and 55 °C. Instead, PCE may have escaped from the glass ampules by the same mechanisms as described for the VOA vials including poor flame-seal integrity, sorption to the glass walls, and diffusion through the borosilicate glass walls. Ampule seal integrity was not suspected to have led to PCE loss given that ampule mass change was less than 2 mg or 0.005% of total ampule mass over the incubation period. To our knowledge the coefficient for the diffusion of PCE or TCE through borosilicate glass has not been determined; however, the coefficient for neon is less than 10^{-10} cm^2/s between the temperatures of 25 and 55 °C (Shelby, 1974) meaning that diffusion of the larger PCE and TCE molecules through the ampule glass walls would not lead to detectable losses over the time period of these experiments. The fact that the concentration of PCE and TCE were greater in ampules incubated at 55 °C as compared to 25 °C suggests that losses from the ampules were in part due to adsorption of these compounds to the glass walls of the ampules, where the higher temperature favored partitioning to the gas and aqueous phases present in the ampules.

4.2.2.6. Practical Implications of VOA Vial Results

Experimental data presented herein strongly suggest that PCE and TCE escaped from the VOA vials by diffusion through the polymer septa, and that the rate of diffusion increased with increasing incubation temperature. The flame-sealed glass ampule results, which should have

minimal loss of PCE and TCE, further support the conclusion that the reductions in PCE and TCE concentrations observed in the VOA vials were due to loss of these compounds through the septa. Therefore, VOA vials sealed with polymer septa are not suitable for the study of PCE and TCE dechlorination reactions at elevated temperatures. These experimental data also serve to emphasize the need to confirm measurements of volatile organic compound degradation with analyses of daughter product formation to demonstrate mass balance closure.

Based on the results obtained for the flame-sealed ampules, the rate of PCE and TCE hydrolysis at 55 °C is slow and would not lead to significant reductions in contaminant mass during thermal treatment. However, these experiments were completed without aquifer solids which may provide additional dechlorination mechanisms. For example, Costanza et al. (2005) reported that the addition of the iron mineral goethite increased the rate of TCE dechlorination by an order of magnitude at 120 °C. Likewise, Truex et al. (2007) suggest that iron minerals present in aquifer solids contributed to the dechlorination of TCE during thermal treatment. While the rate of TCE dechlorination has been show to increase when reactive solids are present, dechlorination of PCE was not increased with the addition of similar reactive solids (Costanza, 2005; Costanza and Pennell, 2006; Costanza et al. 2007a).

4.2.3. Effects of Ion Species and Strength on TCE and PCE Degradation

These experiments involved 40 ampules that contained TCE dissolved in anoxic water at 100 mg/L with argon in the ampule headspace. Five ampules were amended ferrous iron (Fe^{2+}) at 3.3 mM, five with manganese (Mn^{2+}) at 3.3 mM, and five ampules sulfide (S^{2-}) at 0.6 mM. These ion solutions were prepared from analytical grade chemicals and added in sufficient concentrations to achieve the complete oxidation (Fe^{2+} and Mn^{2+}) or reduction (S^{2-}) of the TCE initially present. Five ampules were un-amended and served as controls. The ampules were incubated at 70 °C for a period of 13 days and then destructively sampled to determine the TCE content along with degradation products. There was a 20% reduction in the amount of TCE in the ampules amended with ferrous iron (Fe^{2+}) and a 10% reduction in ampules with sulfide (S^{2-}) as compared to the amount of TCE in ampules in un-amended ampules. There was no change in the TCE content of ampules amended with manganese (Mn^{2+}). The decrease in TCE content for the ferrous iron and sulfide containing ampules was matched with increases in chloride, formate, CO, and CO_2 levels, which are compounds that indicate thermally induced abiotic degradation of TCE. Thus, TCE degradation was increased at the temperature of 70 °C with the addition of ferrous iron and sulfide. These preliminary results provide justification for additional studies to investigate the effects of ionic compounds on abiotic degradation of TCE and PCE.

A follow-on experiment was initiated based on the finding that ferrous iron was detected in groundwater samples received from the Camelot Cleaners Superfund site. The experiment is being performed to determine if the ferrous iron in these field samples would lead to the degradation of TCE and PCE. Ampules were filled with 15 mL of groundwater within an argon filled chamber and then spiked with a methanol stock containing TCE and PCE to create initial concentrations of 10 mg/L. There were 6 ampules prepared from each of 12 groundwater samples (72 ampules) that represented 4 monitoring wells and depths from 7 to 56 feet bgs. All samples contained ferrous iron at initial concentrations of greater than 100 mg/L and one sample contained sulfide at greater than 50 mg/L in addition to iron. Of the 72 ampules, 36 were incubated at 70 °C, and 36 were stored at 25 °C to serve as controls.

The ampules that contained TCE and PCE spiked groundwater from the Camelot Cleaners site collected from monitoring well PMW1 at 16, 27, 45, and 58 feet bgs were destructively sampled after 33 days of incubation at 25 and 70 ^{o}C. There were slight decreases (~10%) in the amount of TCE and PCE in ampules with groundwater from 16 and 58 feet bgs that were incubated at 70 ^{o}C as compared to the amount in paired ampules stored in the dark at 25°C. The decreases of PCE and TCE coincided with decreases in ferrous iron content and, in the case of the ampules prepared with water from 58 feet bgs, with increases in ferric iron content. The amount of CO and CO_2 increased in the ampules with groundwater from 16 and 58 feet bgs demonstrating that oxidation of TCE and PCE is possible under reducing conditions where ferrous iron was present. Currently, there are 48 ampules with groundwater from Camelot wells that have been at 70 ^{o}C for the past 121 days and ampules with groundwater collected from monitoring well PMW2 at 7, 27, 37, 47, and 58 feet bgs will be destructively sampled during the upcoming quarter.

The ampules that contained TCE and PCE spiked groundwater from the Camelot Cleaners site collected from monitoring well PMW2 at 7 and 47 feet bgs were destructively sampled after 128 days of incubation. There was a 12% decrease in the amount of TCE and a 24% decrease in the PCE content in ampules that were incubated at 70 °C as compared to the amount in paired ampules stored in the dark at 25 ^{o}C. The decreases of PCE and TCE coincided with decreases in ferrous iron content and increases in ferric iron content. In the ampules with PMW2 groundwater from 7 feet bgs, only ferrous iron was present in the 25 ^{o}C ampules while ferrous iron levels decreased to 40% of the total iron present in the ampules incubated at 70 ^{o}C. These results further support the idea that the oxidation of ferrous to ferric iron may play a role in the degradation of TCE and PCE during thermal treatment.

4.2.4. Reactivity of TCE in Ampules Containing Ottawa sand and Goethite

Previous ampule experiments were conducted at 22 and 120°C for periods ranging from 4 to 40 days to determine the effects of solid phase composition and oxygen content on the thermal induced degradation of TCE (Costanza et al., 2005). For all treatments, no more than 15% of the initial 100 mg/L of TCE was degraded, resulting in the formation of several non-chlorinated products including chloride, CO_2, carbon monoxide, glycolate, and formate. First-order rate coefficients for TCE disappearance ranged from 1.2 to 6.2×10^{-3} 1/day at 120 °C, and were not dependent upon oxygen content or the presence of Ottawa sand. However, the rate of TCE disappearance at 120 °C increased by more than one order-of-magnitude (1.6 to 5.3×10^{-2} 1/day), corresponding to a half-life of 13-44 days for ampules containing 1% (wt.) of the iron mineral goethite and Ottawa sand. These results indicated that the rate of TCE degradation in heated, three-phase systems is relatively insensitive to oxygen content, but may increase substantially in the presence of iron bearing minerals. This section covers subsequent work completed to determine the mechanism by which goethite increases the rate of thermal induced TCE degradation and involved incubating TCE with 6 g of Ottawa sand and goethite (1% wt.) at temperatures of 25 and 120 °C for periods ranging from 2 to 288 days.
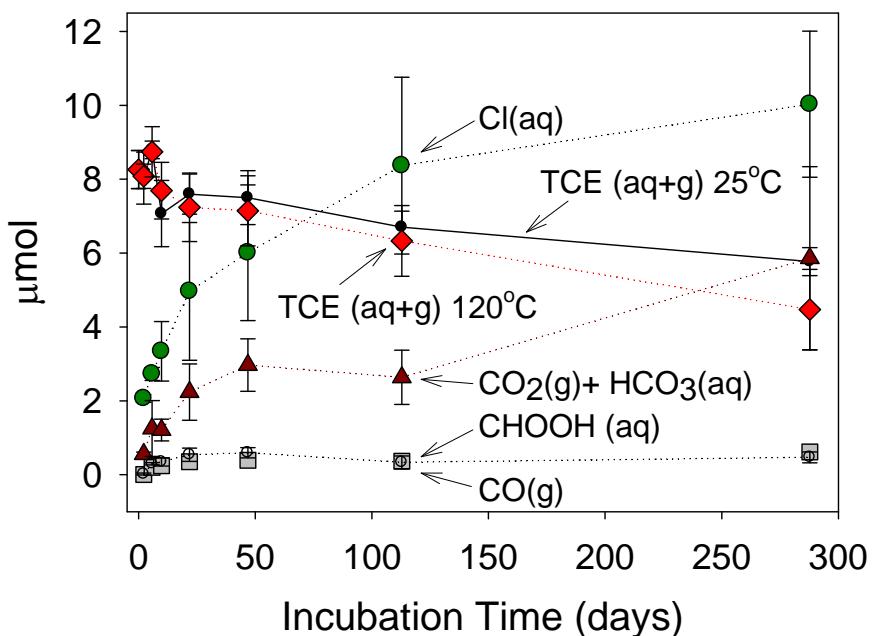
Figure 4-21. Amounts of TCE, chloride (Cl), carbon dioxide (CO_2) corrected for carbonic acid (H_2CO_3), formate ($HCOO^-$), and carbon monoxide (CO) in ampules that contained Ottawa sand and goethite (1% wt.), and anoxic deionized water incubated at 120 °C. Each data point represents the average of five ampules and error bars correspond to one standard deviation.

Figure 4-21 shows the amounts of TCE as a function of incubation time and temperature along with the amounts of TCE degradation products found in the ampules incubated at 120 °C. The experiment involved 55 ampules with 11 ampules per incubation period: 5 with TCE and 3 without TCE (TCE-free) that were incubated at 120 °C, and 3 with TCE maintained at 25°C. Although there was no apparent difference between the amount of TCE in ampules stored at 25 °C and incubated at 120 °C, the amounts of degradation products increased with time in ampules incubated at 120 °C. Chloride and CO_2 were the most abundant products observed, and were also found in TCE-free ampules with Ottawa sand and goethite (1% wt.) that were incubated at 120 °C (data not shown). While carbon monoxide and formate were less abundant than chloride and CO_2, they were only found in ampules containing TCE that were incubated at 120 °C, and therefore, represent compounds that were indicators of TCE degradation in these ampule experiments.

The rate of TCE degradation in experiments with 6 g of Ottawa sand and goethite (1% wt.) was best described by first-order kinetics; however, the rate was not constant over the 288 day incubation period. Over the initial 47 days, the first-order half-life was 164 days based on the appearance of chloride and 168 days based on the appearance of carbon containing reaction products (e.g., carbon monoxide) after correcting for background chloride and carbon levels found in the TCE-free ampules. Between 47 and 288 days, the rate of TCE degradation decreased with a first-order half-life of 682 days based on the appearance of chloride and 459 days based on the appearance of carbon reaction products. The first-order half-life for the

previous experiments was between 13 and 44 days for ampules containing 20 g of same solids incubated over period of up to 40 days at 120 °C (Costanza et al., 2005). Thus, there was a ca. 4 to 13 times decrease in rate of TCE degradation with a faster rate determined in ampules with 20 g of solids vs. the ampules which contained 6 g of the identical solids. The decrease in TCE degradation rate between experiments was attributed to the decrease in solids mass and/or surface area.

Although no reaction mechanism has been elucidated to describe the increase in rate of TCE degradation caused by the addition of goethite to Ottawa sand, it was noted that the rate of sulfate production also increased in ampules that contained TCE relative to TCE-free ampules at 120 °C and those incubated at 25 °C (Figure 4-22). To further investigate this potential mechanism, the mineral content of Ottawa sand was determined using acid extraction followed by metals analysis and by X-ray diffraction techniques to determine the source of sulfate.
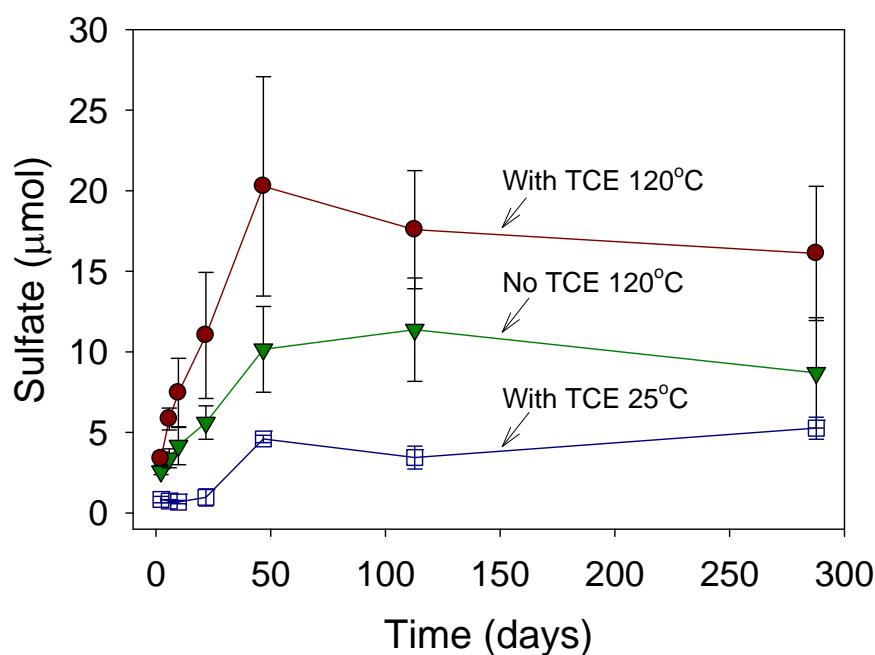
Figure 4-22. Production of sulfate in ampules with 6 g of solids incubated at 25 and 120 $^{\circ}$C.

As anticipated, silica was the predominant element detected in Ottawa sand, while iron was the major component in Ottawa sand amended with 1 wt% goethite (Table 4-3). Unfortunately, the sulfur content of Ottawa sand cannot be determined using the acid extraction technique. To identify the mineral phases present, a sample of acid-treated Ottawa sand was sent to the U.S. Silica Co. Laboratory in Berkeley Springs, WV for X-ray diffraction (XRD) analysis. The mineral phases identified in the sand included pyrite (FeS_2), marcasite (polymorph of pyrite), and hematite (Fe_2O_3). Sulfate was hypothesized to have formed from the oxidation of pyrite and marcasite, or potentially from $CaSO_4$ given that calcium was present in Ottawa sand (Table 4-3).

Table 4-3. Elements present in acid extract of Ottawa sand samples.

Element	Extraction Blank (mg/kg)	Ottawa Sand (mg/kg)	Ottawa Sand + 1 wt% goethite (mg/kg)
Silica	0.4	4.5	11.3
Iron	0.3	3.2	119.4
Calcium	2.5	4.0	32.3

The mechanism of sulfate production during pyrite oxidation requires molecular oxygen (O_2) and/or ferric iron (Fe^{3+}), and also yields ferrous iron (Fe^{2+}) (Stumm and Morgan, 1996). Both the 20 g and 6 g solids experiments were completed using ampules in which the oxygen content had been reduced to approximately 0.3 µmol. However, the concentration of iron was not determined during the experiment completed with 20 g of solids. The experiment conducted for this work incorporated the measurement of ferric and ferrous iron as well as sulfate in order to determine if pyrite was the source of sulfate. While sulfate was produced during the experiment with 6 g of solids (Figure 4.22), neither ferric nor ferrous iron concentrations exceeded the 1 mg/L detection limit. The pH in these ampules was approximately 3.0, and therefore dissolved iron was expected to be stable and should have been detected if present. Theoretically, ferrous iron concentrations could reach approximately 600 mg/L after 47 days of heating at 120 °C if the pyrite was completely oxidized by ferric iron. However, given that TCE was also oxidized in these low oxygen content ampules, it was hypothesized that a heat-activated sulfur oxidant was present in the Ottawa sand or goethite.

A subsequent experiment was undertaken to determine if Ottawa sand contained sulfur oxidants such as persulfate ($S_2O_8^{2-}$). The experiment consisted of measuring the amount of sulfate formed in 40 mL vials that contained Ottawa sand and aqueous solutions of the transition metals Ag^+ and Co^{2+}. These transition metals are known to induce sulfur oxidants to react, for which the rate of sulfate production is dependent on the transition metal used (Anipsitakis and Dionysiou, 2004). The measured sulfate concentrations after 22 days at room temperature (25 °C) increased with an increase in Ottawa sand mass, and were greatest in vials that contained only DI water (Figure 4.23). Sulfate was also produced in vials that contained Ottawa sand that had not been acid washed and dried (untreated sand), indicating that sulfate production was not enhanced by the sand pretreatment process, which included heating the sand in air at 200 °C for 2h. The results shown in Figure 4-23 indicate that increasing ionic strength resulted in a decrease in sulfate production. This result, combined with the slow rate of sulfate formation, suggests that the source of sulfate was the FeS_2 that naturally occurs in Ottawa sand, rather than $CaSO_4$ or sulfur oxidants.
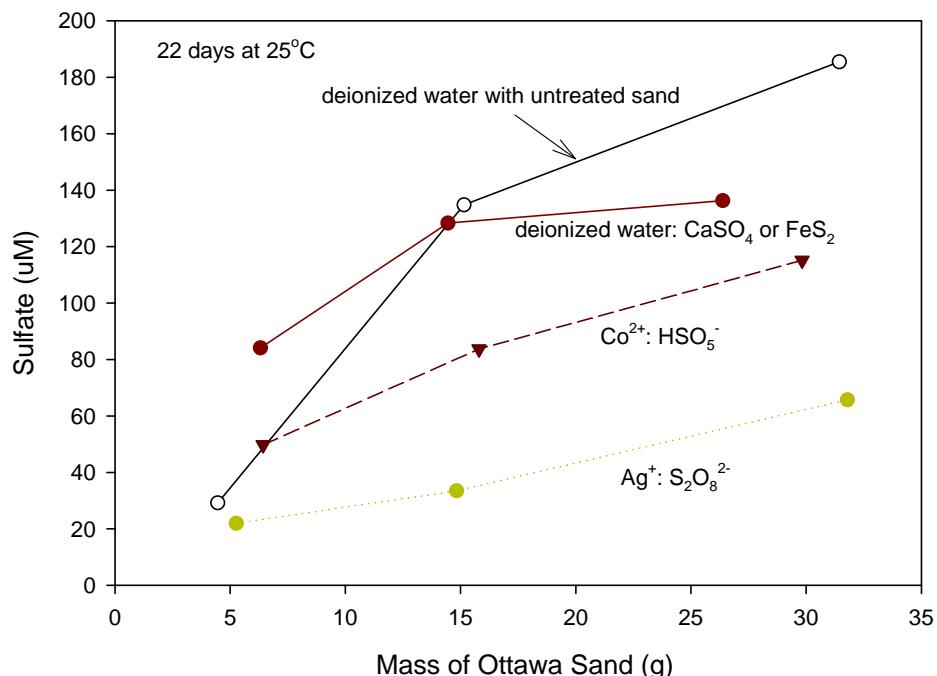
Figure 4-23. Production of sulfate from Ottawa sand in aqueous solutions after 22 days at 25 °C.

The cessation of sulfate production after 47 days (Figure 4.22), which also coincided with the decrease in the rate of TCE degradation, and the increase in sulfate levels for TCE containing ampules suggests that there was a relationship between pyrite oxidation and TCE oxidation. The link between the two may have been related to the lack of dissolved iron and the appearance of an orange colored solid, which is indicative of a freshly precipitation iron oxide according to the following formula:

$$Fe^{2+} + 2H_2O \rightarrow FeOOH + 3H^+ + e^- \qquad (4.5)$$

This suggests that as ferrous iron (Fe^{2+}) was formed during pyrite oxidation, it was in tern being oxidized to form an iron oxide mineral (FeOOH) along with excess electrons which may have been the source of reactivity. Thus, if oxidation of pyrite was the initial step in causing TCE oxidation, then the amount of pyrite in Ottawa sand would be expected to be decreasing as it was depleted with incubation time. To demonstrate that the depletion of pyrite was occurring, 50 mL of 1M ferric chloride solution was added to Ottawa sand samples that were recovered after incubating for 2, 10, 47, and 112 days and the amount of ferrous iron formed was determined using the ferrozine method. The amount of ferrous iron formed by ferric chloride oxidation decreased with incubation time corresponding to increases in the amount of sulfate formed (Figure 4-24).

77
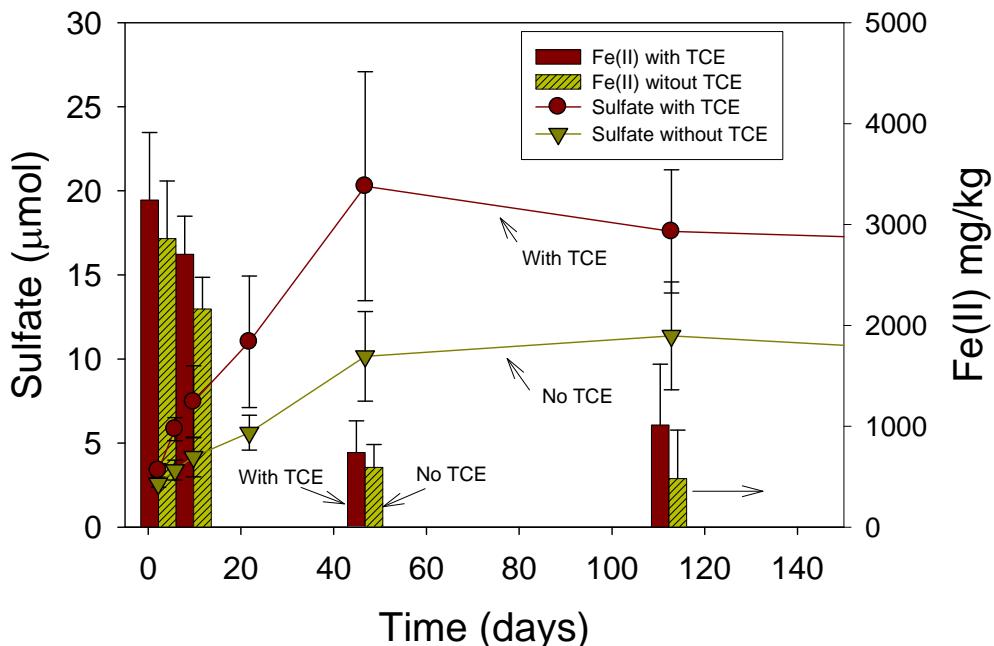
Figure 4-24. Amount of ferrous iron formed by ferric chloride oxidation of Ottawa sand samples after 2, 10, 47, and 112 days of incubation.

The decrease in ferrous iron with incubation time supports the theory that pyrite oxidation was the initial step in the TCE oxidation mechanism. Given that TCE was oxidized rather than reduced during the oxidation of ferrous iron (Equation 4.5), there must have been additional reaction steps involving the formation of oxygen containing reactants such as peroxides. This observation suggests that a Fenton type reaction may have occurred as the ferrous iron was oxidized to ferric iron at 120 °C according to the following:

$$Fe^{2+} + H_2O_2 + H^+ \rightarrow Fe^{3+} + {}^{\bullet}OH + H_2O \qquad (4.6)$$

However, no hydrogen peroxide (H_2O_2) was added to these ampules. One potential in situ source of hydrogen peroxide may have been the pyrite given that the addition of pyrite to oxygen-free water was shown to result in the formation of hydrogen peroxide (Borda et al., 2003). The ferrous iron formed during pyrite dissolution may have reacted with the hydrogen peroxide to form ferric iron, hydroxyl radicals, and hydroxide ions. Thus, the oxidation of TCE in the low oxygen content ampules could have been caused by reaction with hydroxyl radicals and hydroxide ions formed during pyrite dissolution rather than a simple hydrolysis reaction. The increase in TCE oxidation rate with goethite present may be explained by noting that goethite is effective at catalyzing hydrogen peroxide into hydroxyl radicals (Kwan and Voelker, 2003).

4.2.4.1. Practical Implications of TCE Reactivity in Presence of Fe-Containing Solids

The results of this work indicate that the pyrite found in Ottawa sand, in combination with the goethite, led to the formation of reactive oxygen species which resulted in the oxidation of TCE. Given that pyrite is one of the most commonly occurring iron minerals (Stumm and

Morgan, 1996), this finding implies that at thermal treatment sites where pyrite is present, such as Naval Air Station North Island, oxidation of TCE may be expected at a rate which is at least an order of magnitude faster than due to hydrolysis.

4.2.5. Fate of PCE in Heated Camelot Soil

This study was performed to determine the rate and extent of chlorinated solvent degradation after heating contaminated soil and groundwater samples collected from the Camelot Cleaners Superfund site located in West Fargo, ND. The site contained PCE contaminated clay soil and the samples were collected prior to treating the site using electrical resistive heating. Soil samples were homogenized, loaded into 25 mL glass-ampules followed by the addition of groundwater, and then flame sealed to create a batch of 110 ampules. The ampules were incubated at 25, 55, 75, and 95 °C and ampule contents were sampled after 5, 17, 37, and 75 days to determine the reaction products and rates as a function of temperature. These data were used to determine the importance of chlorinated solvent degradation during thermal treatment of the field site.

4.2.5.1. Thermally-Enhanced PCE Recovery

Heating ampules containing Camelot soil and groundwater to 55, 75 and 95 °C resulted in increased aqueous-phase concentrations of PCE (Figure 4-25). In contrast, PCE concentrations remained steady in ampules maintained at 25 °C (1.4 ± 0.8 mg/L, n = 25), including those amended with 9.1 mM of mercuric chloride, which served as the abiotic control. Compared to the PCE concentrations found in the 25 °C control ampules, aqueous PCE levels increased by 3, 4, and 6 times for ampules incubated at 55, 75, and 95 °C, respectively, after 37 days of heating. These results indicate that heating the ampules to temperatures greater than 55 °C caused PCE mass to transfer from the solid to the aqueous phase. This observation supports the suggestion that raising subsurface temperature can increase the rate of chlorinated solvent recovery by increasing concentrations in the extractable aqueous and gas phases (Davis, 1997).

In ampules heated to 55 °C, the concentration of PCE was steady over 37 days, and then decreased after 75 days of heating. For both the 75 and 95°C treatments, PCE concentrations steadily increased with time up to 37 days. While PCE concentrations in the 75 °C ampules after 37 and 75 days of heating remained constant, a statistically significant ($p < 0.05$) decrease in PCE concentration was observed in the 95 °C ampules at 75 days, which was less pronounced in ampules amended with mercuric chloride (abiotic control).
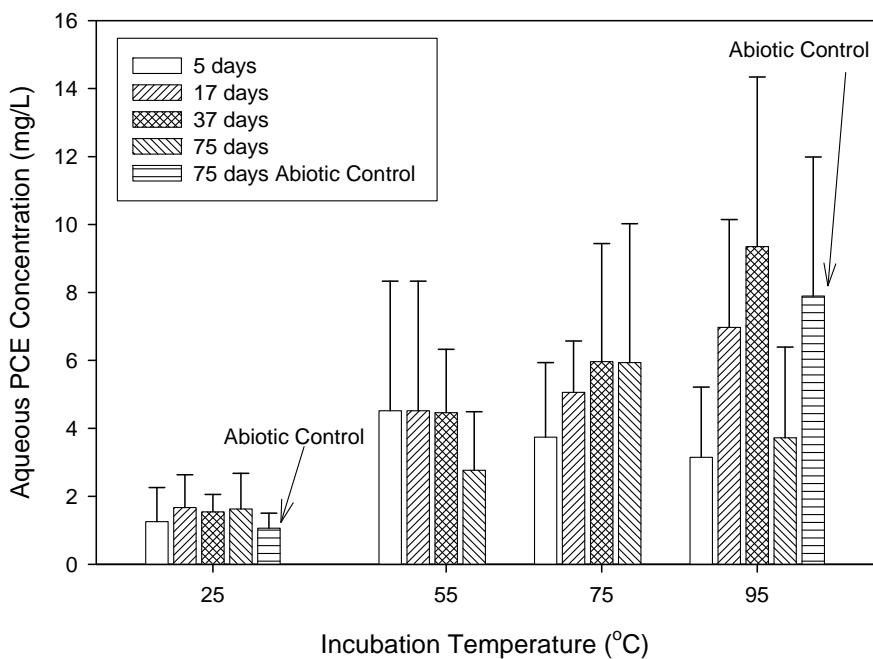
Figure 4-25. Concentration of PCE in the aqueous phase of Camelot ampules. Each bar represents the average of five ampules with error bars equal to one standard deviation.
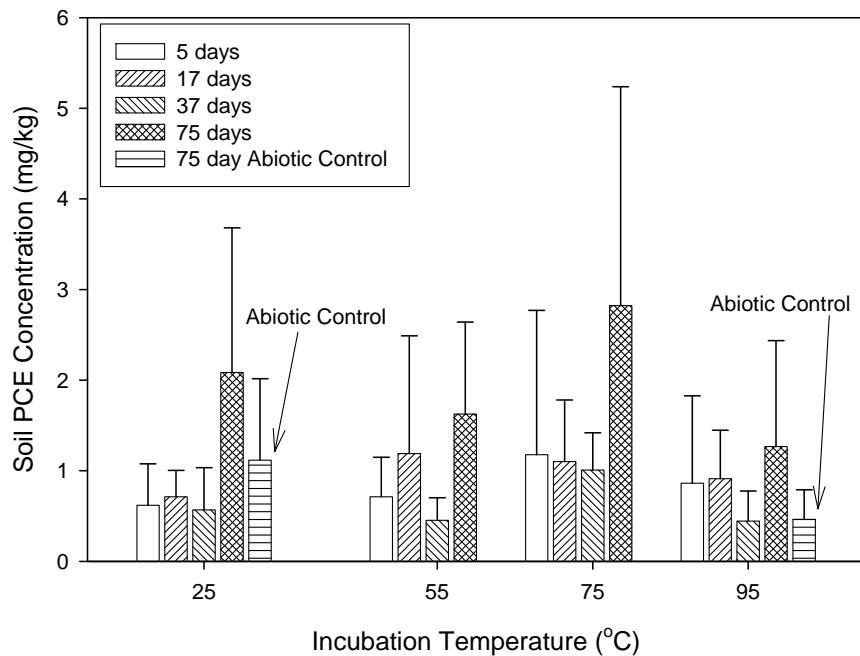
Figure 4-26. Concentration of PCE in the solid phase of Camelot ampules. Each bar represents the average of five ampules with error bars equal to one standard deviation.

While aqueous phase PCE concentrations increased with incubation temperature (Figure 4-25), there was no statistical change in solid phase PCE concentrations with temperature or incubation time ($p > 0.05$, Figure 4-26). The fact that the aqueous phase PCE concentrations increased with incubation temperature while the solid-phase concentrations remained relatively constant suggests that PCE was strongly sorbed by the Camelot soil. Since the soil was air-dried prior to loading in the ampules, the readily desorbable fraction of PCE was likely to have been removed, leaving behind a more strongly sorbed or slow-desorbing PCE fraction (Li and Worth, 2004). The total amount of PCE initially present in the 25 °C ampules was estimated to be 0.75 ± 0.08 µmol based on the mass of soil added to each ampule and the average concentration of PCE determined in methanol extracts from 12 randomly selected *air-dried* soil samples that were collected while loading the ampules. The total amount of PCE detected in the ampules after incubation at 25 °C, as determined from aqueous samples and methanol extracts of *water-wet* soil, was 0.24 ± 0.14 µmol (n = 25), which represents a PCE recovery of 32%. Tetrachloroethylene recovery increased with incubation temperature, ranging from 39 to 83% for ampules incubated at 55 °C, 66 to 120% for ampules at 75 °C, and 72 to 155% for ampules at 95 °C. The increase in PCE recovery with incubation temperature and poor recovery at 25 °C indicates that PCE was sorbed by a fraction of the Camelot soil that was not interrogated by methanol under water-wet conditions.

Table 4-4. Properties of air-dried homogenized soil from the Camelot Cleaners Superfund site.

Total Carbon[a]	Total Nitrogen[a]	Inorganic Carbon as CO_2[b]	Ca^{2+}[c]	Mg^{2+}	K^+	Carbon as Dolomite $CaMg(CO_3)_2$
			Weight Percent			
1.11 ± 0.03	0.06 ± 0.00	1.58 ± 0.30	0.29 ± 0.01	0.26 ± 0.02	0.02 ± 0.0	0.64 to 0.94

[a]dry combustion method performed by the Stable Isotope/Soil Biology Laboratory, Institute of Ecology, The University of Georgia, Athens, GA. [b]based on the volume of CO_2 produced at 22°C after mixing with 6N HCl and 5% $FeSO_4$ for 30 min (Loeppert and Suarez, 1996). [c]Elemental analysis using ICP-MS (Thermo Jarrell-Ash, Enviro 36) after a double acid digest; performed by the Chemical Analysis Laboratory, The University of Georgia, Athens, GA.

The soil sample collected from 45 feet bgs at the Camelot site contained oxidizable carbon and nitrogen based on a dry combustion analysis (Table 4-4); however, the soil produced a similar amount of carbon as CO_2 after addition of a strong acid (Inorganic Carbon, Table 2). The concentrations of calcium and magnesium detected were consistent with the presence of dolomite [$CaMg(CO_3)_2$], and suggest that this was the source of CO_2 in the dry combustion and acid dissolution analyses. The Camelot soil consisted of clay and silt size fractions, with 33% clay (<0.2 µm) and 67% silt (0.2 to 50 µm) size fractions by weight. The clay minerals present included smectite, kaolinite, and illite based on Cu X-ray diffraction (XRD) analysis after dithionite-citrate-bicarbonate (DCB) treatment (Jackson, 2005). The smectite had an interlayer

spacing of 1.5 nm when saturated with magnesium, which collapsed to 1.25 nm upon potassium saturation. Thus, only trace levels of organic carbon existed in the Camelot soil and mineral surfaces including dolomite and clays are suspected to be the dominant source of sorptive surfaces.

The figure shows a scatter plot with Gas Phase CO_2 (%) on the x-axis and Aqueous Phase PCE (mg/L) on the y-axis, with data points for 25°C, 55°C, 75°C, and 95°C, and a linear regression line. Annotated equation:

$$PCE = CO_2 \times 0.98 + 1.65 \quad r^2 = 0.87$$
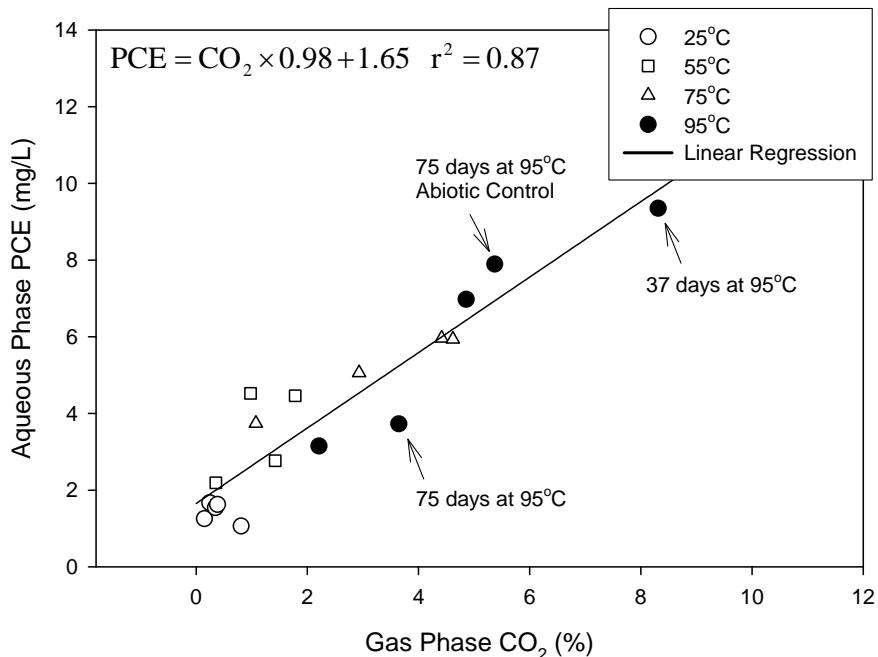
Figure 4-27. Correlation between aqueous phase PCE and gas phase CO_2 concentrations in ampules with Camelot soil and groundwater.

The hypothesis that PCE was sorbed by the Camelot soil mineral phase is supported by the linear relationship between aqueous phase PCE and gas phase CO_2 concentrations found in the ampules (Figure 4-27). Carbonate minerals are suspected to be the primary source of CO_2 because the oxygen required to transform organic carbon into CO_2 was in limited supply; the ampule headspace consisted of argon gas and dissolved oxygen levels were less than 2 mg/L. The similarity between CO_2 production and PCE levels with increasing incubation temperature suggests that the conversion of carbonate minerals to CO_2 released sorbed-phase PCE, which then appeared in the ampule aqueous phase. While the amount of CO_2 detected was substantial, up to 9% of the ampule gas volume (Figure 4-27), this amount represented less than 1% of the mineral CO_2 content present in each ampule (Table 4-4).

We therefore hypothesize that the primary mechanism responsible for the thermally-enhanced mass transfer of PCE from the solid to aqueous phase involved the destruction of carbonate mineral structure that contained sorbed-phase PCE. Additionally, exchange of native monovalent cations on clay mineral surfaces by calcium or magnesium ions could have reduced the sorptive capacity of the Camelot soil. A similar effect of exchangeable cation species on TCE adsorption by smectite clay has been recently demonstrated (Aggarwal et al., 2006).

4.2.5.2. Abiotic Thermal Degradation of PCE in Camelot Soil

The decrease in average PCE concentration after 75 days of heating at 95°C relative to the concentration at 37 days (Figure 4-25) suggests that PCE was degraded during thermal incubation. The similarity in the amount of PCE detected in the room temperature ampoules, no significant difference between the mean amounts of PCE at each sampling interval for the 25 ampules maintained at 25 $^{\circ}$C ($p > 0.05$), suggests that the decrease in PCE content between 37 and 75 days at 95 °C was the result of a degradation process, rather than variability in the initial PCE content. However, the average CO_2 content in ampules incubated at 95 °C for 75 days was less than that observed after 37 days at 95 °C, and in abiotic control ampules amended with mercuric chloride (Figure 4-27). These data suggest that there was either less PCE initially present in the 75-day ampules incubated at 95 °C, or that the PCE-containing carbonate minerals were only partially degraded in the 75 day ampules as compared to the 37 day ampules.

Chloride levels have been used to estimate rates of TCE degradation in ampule experiments completed with low-chloride DI water (Costanza et al., 2005). The chloride levels in the Camelot ampules were ca. 300 mg/L, approximately 50 times greater than the amount of PCE (as chloride) initially present. Therefore, native chloride levels obscured the relatively small increases in chloride content that could be attributed to PCE degradation. Alternatively, CO, a product found during thermal degradation of TCE (Costanza et al., 2005), could prove useful for monitoring PCE degradation. In fact, CO levels steadily increased in Camelot ampules incubated at 95 °C (Figure 4-28). Volatile organic compounds, in addition to PCE, were detected in the gas and aqueous-phase samples collected after 75 days of heating at 95 °C. These compounds included *cis*-1,2-dichloroethylene (*cis*-DCE), trans-1,2-dichlorothylene (*trans*-DCE), TCE, 1-butene, benzene, and furan. Furan was identified based on its mass spectrum, while the other compounds were identified by their mass spectrum and capillary column retention time. Several of these compounds, including TCE, cis-DCE, and 1-butene have been detected as products from the reductive dechlorination of PCE in experiments conducted with zero valent iron (Arnold and Roberts, 2000).

An additional experiment was conducted to evaluate the source of CO and 1-butene using PCE-free soil collected from 56 feet bgs at the Camelot site. Twenty-four ampules were prepared with the PCE-free Camelot soil and deionized water, and then incubated at 25 and 95 °C for 75 days. The compounds detected in the PCE-free ampules included CO, CO_2, 1-butene, and furan, with a small amount of benzene after 75 days at 95 °C. The amounts of CO formed in these PCE-free ampules were similar ($p > 0.05$) to those found in the ampules that contained PCE (Figure 4-28). Thus, CO production was attributed to soil diagenesis, rather than PCE degradation in ampules heated to 95°C.
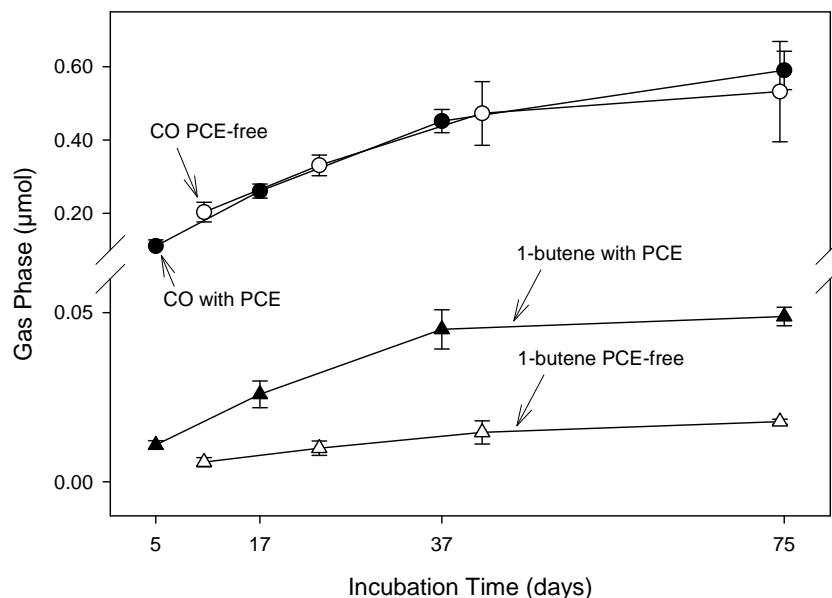
Figure 4-28. Amounts of CO and 1-butene in ampules incubated at 95 °C with PCE or PCE-free Camelot soil. Each data point represents the average of five ampules with error bars equal to one standard deviation.

Carbon dioxide formed during in-situ thermal treatment has also been suggested to represent the destruction of chlorinated solvents (Knauss et al., 1999). The concentration of CO_2 in the PCE-free ampules incubated at 95°C for 75 days was 3.6 times greater than in the ampules that contained PCE-contaminated Camelot soil and groundwater. Thus, the CO_2 observed in ampules with PCE contaminated Camelot soil was indicative of soil carbon and carbonate degradation, rather than degradation of chlorinated solvents.

While the amounts of CO detected in PCE-contaminated and PCE-free ampules heated to 95 °C were similar (Figure 4-28), more 1-butene was detected in ampules containing PCE compared to the PCE-free ampules. The difference in the amounts of 1-butene may have been due to PCE degradation or to differences in the aqueous-phase composition of ampules since the PCE-free ampules were filled with deionized water, while the ampules with PCE contained groundwater from the Camelot site. That is, the Camelot groundwater may have contained additional precursors necessary for 1-butene formation. Regardless of the formation pathway, the fact that 1-butene was detected in ampules with PCE-free Camelot soil renders this compound a poor indicator of PCE degradation under these conditions. The observation of 1-butene and furan in the PCE-free ampules demonstrates that soil organic carbon was present in the Camelot soil. However, the levels of carbon as CO_2 were 400 to 800 times greater than the amount of carbon associated with 1-butene and furan, which supports the conclusion that there was little organic matter in the Camelot soil.

Trichloroethylene was detected in the aqueous phase of ampules containing PCE-contaminated Camelot soil incubated at 55, 75, and 95 °C (Figure 4-29) and may represent a PCE degradation product. Alternatively, TCE may have been present in the Camelot soil from 45

feet bgs, but was only released as the carbonate in the soil was thermally degraded. In either case, the amount of TCE decreased with incubation time, with the greatest degradation rate in ampules heated to 95 °C. First-order rate coefficients (k), shown in Figure 4-29, increased with incubation temperature indicating that the degradation followed the Arrhenius equation:

$$k = A\exp\left(\frac{-E_a}{RT}\right) \tag{4.7}$$

where A is the pre-exponential factor, E_a is the activation energy, R is the gas constant, and T is absolute temperature. A plot of $\ln(-k)$ vs. $1/T$ (not shown) yielded an activation energy (E_a) of 44.5 kJ/mol. This activation energy is similar to that reported for the degradation of TCE in a heated enclosure with only an aqueous-phase present (Knauss et al., 1999). However, the pre-exponential factor (A) was reduced by greater than 500 times, indicating that the presence of a solid phase (Camelot soil) and gas phase decreased the rate of TCE degradation.
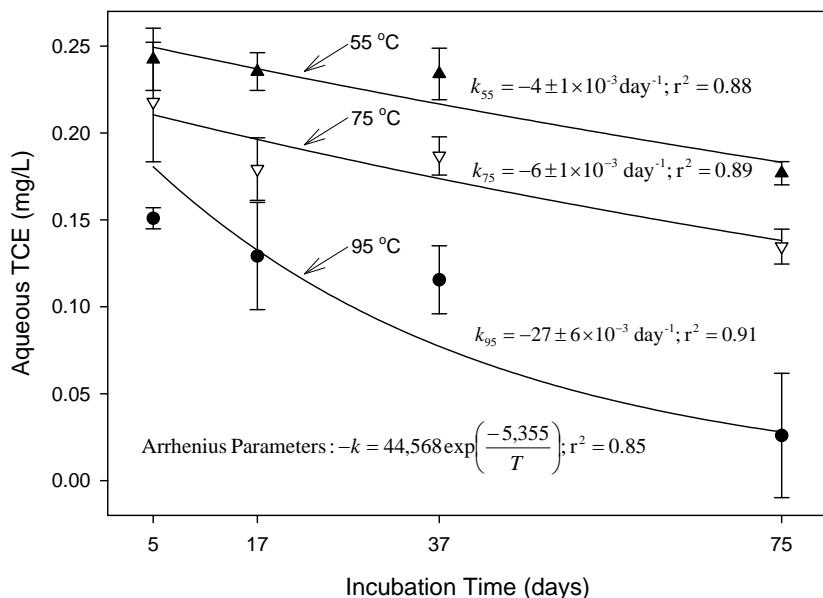
Figure 4-29. Concentration of TCE in the aqueous phase of ampules with PCE contaminated Camelot soil. Solid lines represent first-order kinetic model fit by linear regression analysis. The first-order rate coefficients (k) and correlation coefficients (r^2) are shown for each incubation temperature. Each data point represents the average of five ampules with error bars equal to one standard deviation.

In ampules incubated at 95 °C, the amount of cis-DCE increased with incubation time, coinciding with a decrease in the amount of TCE (Figure 4-30). This finding suggests that TCE underwent abiotic reductive dechlorination since a similar amount of cis-DCE was also noted in ampules amended with mercuric chloride (abiotic control). The degradation of TCE to cis-DCE was evident only in the 95 °C ampules, as no cis-DCE was detected in the ampules incubated at 55 or 75 °C. The observed transformation of TCE (C_2HCl_3) to cis-DCE ($C_2H_2Cl_2$) requires a source of hydrogen as described by:

$$C_2HCl_3 + H_2 \xrightarrow{\Delta} C_2H_2Cl_2 + HCl \tag{4.8}$$
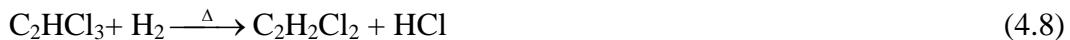
The level of hydrogen (37.1 $\pm$ 12.6 ppmv) in the gas phase of the Camelot ampules incubated at 95 °C for 75 days was approximately 18 times greater than in ampules incubated at lower temperatures. The presence of the additional amount of hydrogen in the 95°C ampules along with the added thermal energy resulted in TCE hydrogenolysis; the addition of hydrogen to TCE with the elimination of Cl to form cis-DCE. This reaction (Eq. 4.8) was best fit using a first-order model that also included an initial amount of cis-DCE (0.017 µmol) as shown in Figure 4-30. Based on the rate coefficients shown in Figure 4-29, the first-order half lives of TCE were 157 days at 55 °C and 26 days at 95 °C. The concentration of other potential reductants, including ferrous iron (Fe^{2+}) and sulfides, were below the method detection limit.
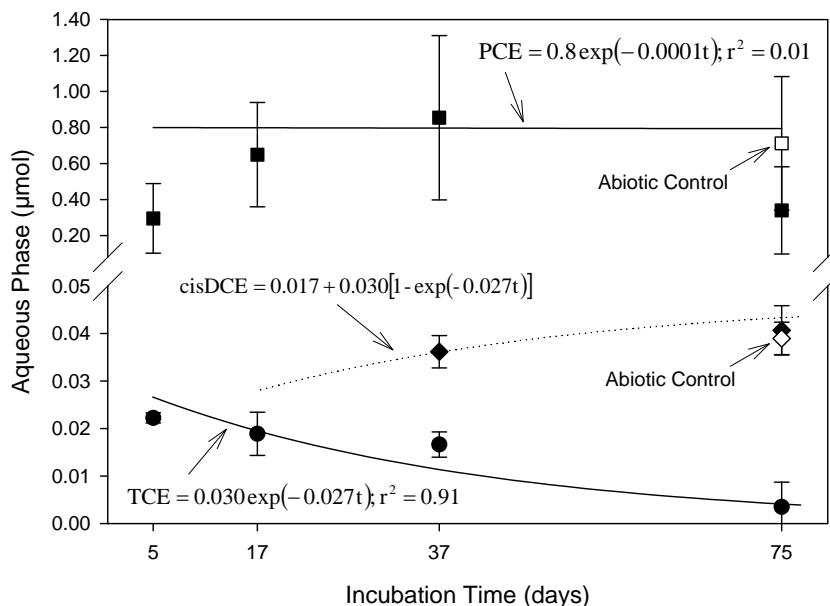
Figure 4-30. Amount of PCE, TCE, and *cis*-DCE in aqueous phase of Camelot ampules incubated at 95 °C. Solid lines represent first-order kinetic model fit by linear regression analysis. Each data point represents the average of five ampules with error bars equal to one standard deviation.

The rate of PCE degradation necessary to fit the observed TCE degradation at 95 °C (Figure 4-30) resulted in a PCE half-life of ca. 7,000 days using a first order kinetic model. Based on this finding, and the fact that PCE levels remained elevated even after 75 days of heating at 95 °C, it is reasonable to conclude that thermally-induced PCE degradation will not be a significant treatment process during electrical resistive heating of the Camelot Cleaners Superfund site. On the other hand, if TCE is present or formed as a reaction product, it would be expected to degrade during treatment with the formation of *cis*-DCE once temperatures approach 95 °C.

4.2.6. Fate of TCE in Heated Fort Lewis

This study was performed to determine the rate and extent of TCE and *cis*-DCE degradation caused by heating contaminated soil and groundwater samples collected from the East Gate Disposal Yard (EGDY) located in Fort Lewis, WA. The soil samples, which were collected prior to thermal treatment by electrical resistive heating, were homogenized and loaded into 59 glass-ampules. Ampules were amended with groundwater and 1.5 µmol of TCE in a methanol stock and then flame sealed. The ampules were incubated at 25, 50, and 95 ^{o}C, and were destructively sampled after 10, 23.6, 42, 66.6, and 95.5 days to determine the concentrations of reaction products and rates of degradation as a function of temperature. The resulting data were used to estimate the fraction of TCE mass that could be degraded during in-situ thermal treatment of the EGDY site.

4.2.6.1. Biotic Degradation of TCE in Ampules

Over the initial 42 days, the amount of TCE in the gas and aqueous phases of solids-containing ampules incubated at 25 °C accounted for only 49±13% of the amount of TCE introduced (Figure 4-31). After 66.6 days, the amount of TCE in the gas and aqueous fractions of the solids-containing ampules decreased to less than the 0.2% of the initial amount, while the TCE content in identically prepared water-only ampules incubated at 25 °C remained constant at 1.03 ± 0.12 µmol over 95.5 days. TCE disappearance from the solids-containing ampules at 66.6 and 95.5 days was accounted for by the formation of *cis*-DCE, which is a product from both abiotic and biotic reductive dechlorination of TCE (Arnold and Roberts, 1998; Sung et al., 2006b). Concomitantly, the amounts of gas-phase CO_2 and CH_4, and aqueous-phase acetate increased in the solids-containing ampules maintained at 25 °C. The nitrate concentration in solids-containing ampules was below the detection limit of 5 µM after 10.4 days as compared to the constant levels of 61 ± 1 µM in the water-only ampules. The sulfate concentration in solids-containing ampules decreased from ca. 80 µM at 10.4 days to 3 µM after 66.6 days. In water-only ampules amended with 10 µL of TCE-methanol stock, there were no measurable changes in gas-phase CO_2 and CH_4 levels, and aqueous-phase acetate, nitrate, sulfate, and chloride levels at 25 °C. These data suggest that acetogenic, nitrate and sulfate reducing, and reductively dechlorinating bacteria and methanogenic archaea were present in the EGDY soil collected from 28 to 30 feet bgs at location RS0047b.
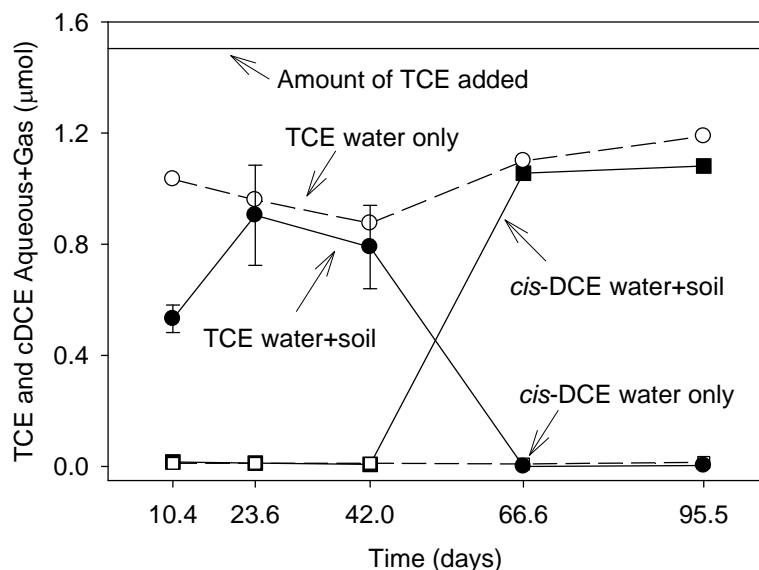
Figure 4-31. Amounts of TCE and *cis*-DCE in aqueous phase of ampules containing EGDY soil and groundwater incubated at 25°C.

A 16S rRNA gene sequence that was highly similar (99% over 1,350 base-pairs) to that of the known TCE-to-*cis*-DCE dechlorinating bacterium *Geobacter lovleyi* (Sung et al., 2006b) was obtained from the clone library constructed from the TCE-dechlorinating enrichment culture. The methanol added with the TCE spike was the only electron donor amendment, which accounted for 250 μmol of methanol or 10 times the amount of CO_2 and acetate detected after 95.5 days at 25 °C. At the conclusion of the experiment, DNA was extracted from 25 °C ampule solids. The number of bacterial 16S rRNA gene copies per gram of soil was 3.5×10^7, while the number of copies for archaeal cells was below the detection limit of 1×10^3. Even though archaeal cells were not detected in the ampule solids, the increasing CH_4 levels after sulfate consumption suggest that methanogenic archaea were active in the solids containing ampules incubated at 25 °C (Costanza et al., 2009).

In ampules incubated at 50 °C, the amount of TCE in the gas and aqueous phases of the solids containing ampules, with respect to the amount introduced, was $56 \pm 7\%$ over 95.5 days, and the amount of *cis*-DCE remained constant at 17.7 ± 7.4 nmol (Figure 4-32). The relatively constant TCE and *cis*-DCE levels coincided with constant CO_2 levels at 1.1 ± 0.3 μmol and nitrate concentrations of 26 ± 5 μM after 95.5 days, and an increase in sulfate concentrations from 99 ± 5 μM after 10.4 days to 164 ± 6 μM after 95.5 days. Increases in sulfate, likely due to mineral dissolution, were previously observed in ampules containing Ottawa sand that were incubated at 120 °C (Costanza et al., 2005). While increases in formate and acetate concentrations were observed, the concentrations were ca. 50 times lower after 42 and 95.5 days compared to the levels found in solids containing ampules incubated at 25 °C. Slight increases in CH_4 concentrations were observed, from below detection limit of 0.006 μmol after 10.4 days, to 0.03 ± 0.2 μmol after 66.6 days, which could have been produced by thermophilic methanogens or formed by heating organic matter present in the soil (Deng and Dixon, 2002). Thus, heating of EGDY soil to 50 °C resulted in decreased microbial activity to the extent that microbial TCE, nitrate, and sulfate reduction were not detected.
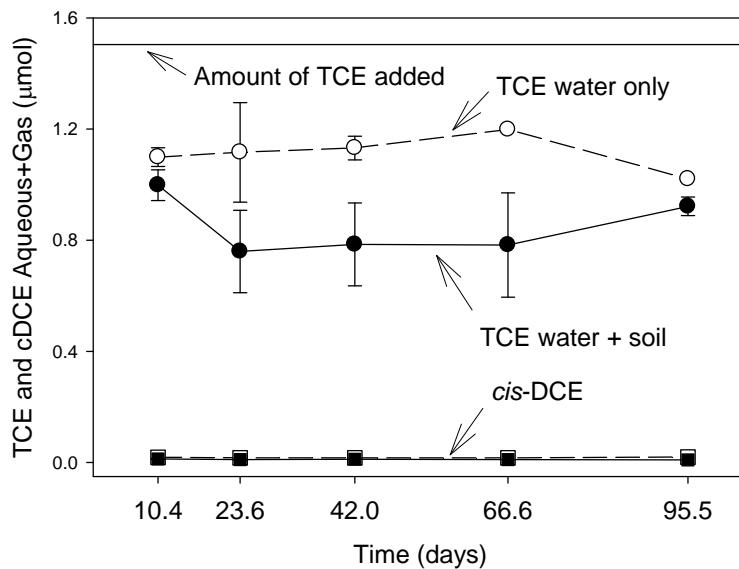
Figure 4-32. Amounts of TCE and *cis*-DCE in aqueous phase of ampules containing EGDY soil and groundwater incubated at 50 °C.

In ampules incubated at 95 °C, the amount of TCE in the gas and aqueous phases of the solids-containing ampules was 79±9%, of the amount introduced over 95.5 days, and *cis*-DCE remained constant at 20.9±6.0 nmol (Figure 4-33). The amount of CO_2 in ampules incubated at 95 °C was constant at 5.3±0.7 μmol as was acetate at 0.4±0.0 μmol over 95.5 days, while the amount of formate increased from 0.2±0.0 μmol after 10.4 days to 1.2±0.5 μmol after 95.5 days. The concentration of nitrate was constant at 31±5 μM over 95.5 days, and sulfate concentrations increased from 127±4 μM after 10.4 days to 216±28 μM after 95.5 days. Methane concentrations were greatest in ampules incubated at 95 °C, compared to ampules incubated at 25 and 50 °C, increasing from 0.03±0.01 μmol after 10.4 days to 0.07±0.04 μmol after 95.5 days. While increases in methane concentrations may be indicative of thermophilic methanogen activity, methane can also be formed by heating soil organic matter (Deng and Dixon, 2002). Because reducing conditions did not develop in EGDY soils heated to 95 °C, as evidenced by the lack of nitrate and sulfate reduction, it is unlikely that methane was produced biotically and therefore, similar to the 50 °C results, heating EGDY soil to 95 °C caused a decrease in microbial activity.
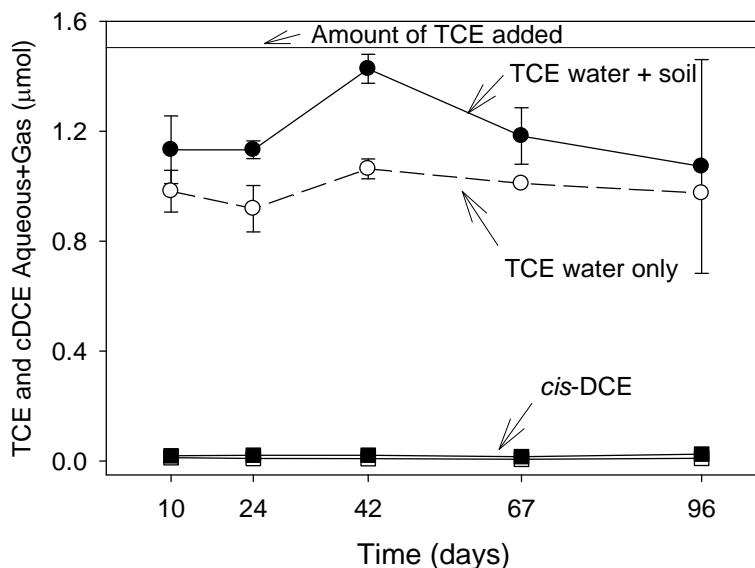
Figure 4-33. Amount of TCE and *cis*-DCE in aqueous phase of ampules containing EGDY soil and groundwater incubated at 95 °C.

These results demonstrate that microbial degradation of TCE was significant at 25°C, but did not occur when the temperature was increased to either 50 or 95 °C, consistent with the findings of Friis et al. (2007) and Truex et al. (2007) for EGDY site solids. The results from the 50 and 95 °C incubations, when compared to the 25°C incubation results, suggest that there were substantial decreases in bacterial activity caused by heating EGDY aquifer material collected from 28 to 30 feet bgs at location RS0047b. While sulfate-reducing bacteria were active during incubation at 25°C, heating this aquifer material prevented sulfate reduction over the 95.5 day incubation period. The number of bacterial 16S rRNA genes per gram of soil decreased from 3.5 $\times$ 10^7 in solid samples obtained from ampules maintained at 25 °C, to 1.7 $\times$ 10^5 at 50 °C and 6.1 $\times$ 10^6 at 95 °C. For all three incubation temperatures, the number of archaeal cells was below the qPCR detection limit of 1 $\times$ 10^3 archaeal 16S rRNA genes per gram of soil. Thus, the decrease in microbial activity coincided with measured decreases in the number of bacterial 16S rRNA genes per gram of soil, with the greatest decrease occurring at 50 °C.

The hypothesis that heating the EGDY aquifer would stimulate thermophilic sulfate reducers resulting in reducing redox conditions was not supported by the results of the present study, and was also not evident from the data reported by Friis et al. (2005). Despite efforts to minimize the exposure of Fort Lewis soils and ampules to oxygen, oxidizing conditions were initially pervasive given that nitrate and sulfate were present. After sealing the ampules, reducing conditions only developed in ampules containing soil that were incubated at 25 °C as indicated by the complete consumption of nitrate after 10.4 days and sulfate after 66.6 days, and the conversion of TCE to *cis*-DCE. However, reducing conditions did not develop in ampules incubated at 50 and 95 °C suggesting that there were an insufficient number of thermophiles present.

4.2.6.2. Abiotic Degradation of TCE in Ampules

Increases in aqueous chloride levels were anticipated in the 25 °C ampules where TCE was completely transformed to *cis*-DCE via microbial reductive dechlorination activity. Chloride levels increased from 2.2 ± 0.3 μmol through 42 days, to 3.0 μmol (n=1) after 95.5 days in ampules maintained at 25 °C (Figure 4-34a). This 0.8 μmol chloride increase was 65.5% of the 1.22 ± 0.07 μmol decrease in TCE mass observed between 42 and 95.5 days. The increase in chloride was more obvious when normalized to the mass of solids in each ampule, and in comparison to chloride levels in TCE-free vials (Figure 4-34b). Chloride increased from 3.55 ± 0.02 mg/kg to 12.0 mg/kg after 95.5 days in TCE-containing ampules incubated at 25 °C, whereas chloride levels remained constant at 5.1 ± 1.3 mg/kg in TCE-free vials over 97.7 days at 25 °C. The chloride levels in the 25 °C water-only ampules also remained constant at 1.39 ± 0.03 μmol over the 95.5 day incubation period. Thus, chloride increases detected in the 25 °C ampules containing EGDY site solids correlated to the loss of TCE due to microbial reductive dechlorination activity.
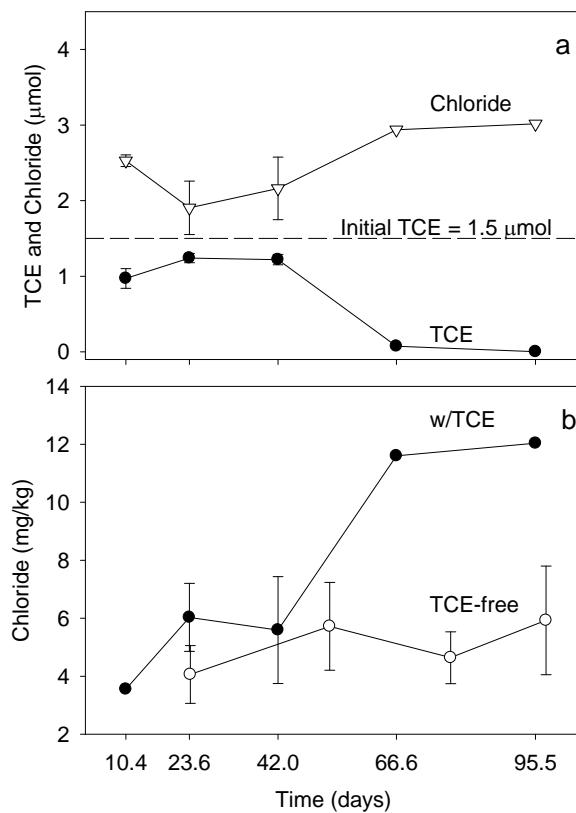
Figure 4-34. Amount of TCE in the gas and aqueous phases along with aqueous chloride in ampules containing EGDY soil and groundwater incubated at 25 °C.

In ampules incubated at 50 °C, chloride amounts increased slightly from 2.1 ± 0.1 μmol after 10.4 days to 2.6 ± 0.5 μmol after 23.6 days, and chloride levels remained constant at 2.6 ± 0.8 μmol thereafter (Figure 4-35a). This increase was less apparent after normalizing for solids content, where the chloride concentration in TCE-containing ampules incubated at 50 °C was

constant at 5.9 ± 1.6 mg/kg (Figure 4-35b). While these chloride levels were 1.1 to 1.9 times greater than those detected in TCE-free vials, none of the differences were statistically significant ($p > 0.05$). The chloride levels in the 50 °C, water-only ampules, also remained constant at 1.44±0.03 μmol over the 95.5 day incubation period. While chloride and TCE levels were relatively stable, the levels of other compounds indicative of TCE degradation, including acetylene, ethene, and ethane, increased over the 95.5 day incubation period. However, the total amount of these compounds detected in the heated ampules represented less than 2.5% of the introduced TCE mass. Truex et al. (2007) also observed these compounds in samples collected during thermal treatment at the EGDY site and attributed their formation to the degradation of TCE by either biotic or abiotic reductive dechlorination. While these compounds may represent TCE degradation, they can also be formed by heating organic matter present in the soil (Deng and Dixon, 2002).
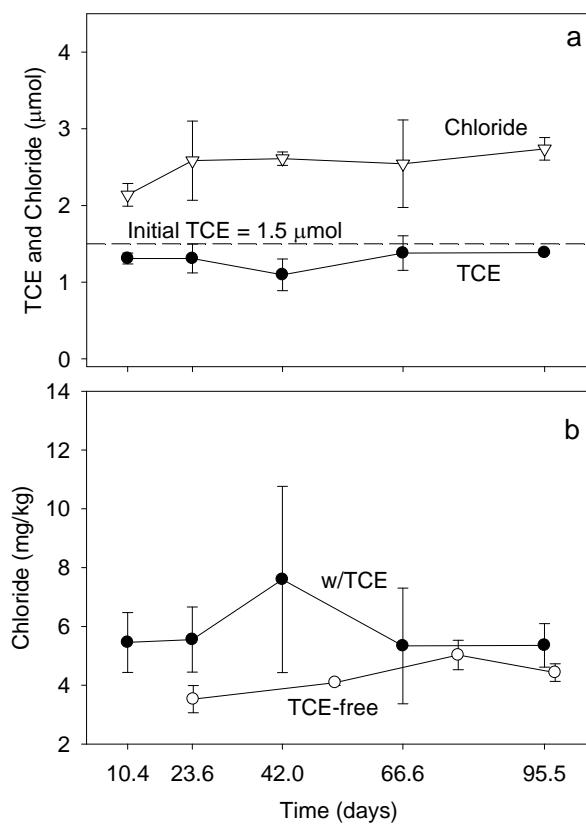
Figure 4-35. Amount of TCE in the gas and aqueous phases along with aqueous chloride in ampules containing EGDY soil and groundwater incubated at 50 °C.

The total amount of TCE detected in ampules incubated at 95 °C was 98 ± 7% of the mass introduced, which was greater than the recovery of TCE from ampules incubated at 25 and 50 °C. Despite this high mass recovery, chloride levels increased with a first-order rate coefficient of $3.5 ± 0.6 \times 10^{-3}$ 1/day based on changes in aqueous phase concentrations (Figure 4-36a) and $3.0 ± 0.3 \times 10^{-3}$ 1/day based on the solids normalized results (Figure 4-36b). These increases in chloride were not apparent in the TCE-free vials, where chloride levels remained

constant at 5.0 ± 0.4 mg/kg over 97.7 days (Figure 4-36), or in the TCE-containing water-only ampules, which exhibited an average chloride level of 1.61 ± 0.09 µmol over 95.5 days. Based on these chloride increases, TCE was dechlorinated in the 95 °C ampules, with a first-order half-life between 1.6 and 1.9 years assuming complete dechlorination (i.e., three chloride ions produced per TCE molecule). In addition to increases in chloride levels, mono- and di-chloroacetylene were detected by GC-MSD analysis of gas samples collected from ampules incubated at 95 °C. Although these compounds were detected at levels of less than 2% of the TCE chromatographic peak area, they are indicative of TCE dechlorination as observed during the oxidation of TCE at 120 °C (Costanza et al., 2005) and during the dechlorination of TCE at ambient temperatures by zero-valent zinc (Arnold and Roberts, 1998). The other potential degradation products including acetylene, ethene, and ethane were also detected in the 95°C ampules; however, the amounts produced were not significantly different ($p > 0.05$) than those detected in the 50°C ampules.
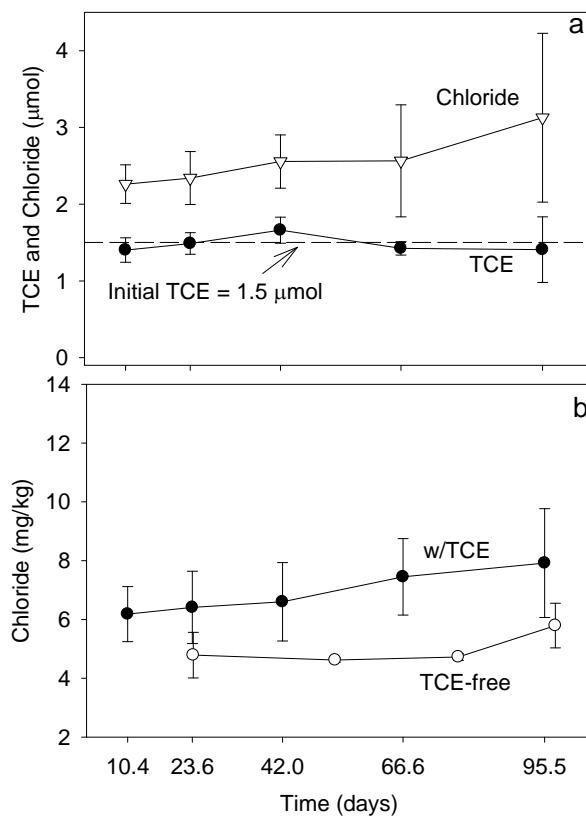
Figure 4-36. Amount of TCE in the gas and aqueous phases along with aqueous chloride in ampules containing EGDY soil and groundwater incubated at 95 °C.

These findings are indicative of slow, abiotic degradation of TCE in EGDY soils incubated at 95 °C, with a first-order half-life for TCE ranging from 1.6 to 1.9 years. In general, these time frames will be greater than those required to recover TCE from the subsurface during in situ thermal treatment through physical processes of mass transfer and gas-phase extraction.

Thus, we conclude that while TCE degradation may occur during thermal treatment of EGDY soil, the reaction rates would not be expected to contribute significantly to mass reductions for electrical resistive heating remediation lasting less than 6 months at temperatures below 95 °C. These results indicate that claims of significant mass destruction due to fortuitous in situ dechlorination during thermal treatment should be viewed with caution and require verification with careful laboratory studies. Such laboratory studies should include choroethene-free soils so that products resulting from heating uncontaminated soil (e.g., acetylene) can be distinguished from chloroethene degradation products (Costanza and Pennell, 2007a). Importantly, these experiments should be conducted in glass ampules rather than polymer sealed vessels to avoid losses of chlorinated ethenes during elevated temperature incubations (Costanza and Pennell, 2007b).

4.2.7. Fate of PCE in Heat Soils from the Great Lakes Naval Training Center

This study was performed to determine the rate and extent of PCE degradation caused by heating contaminated soil and groundwater samples collected from the Naval Training Center Great Lakes located in Great Lakes, IL. The soil samples, which were collected prior to thermal treatment by electrical resistive heating, were homogenized and loaded into 88 glass ampules and then flame sealed. The initial aqueous phase concentrations of 50 mg/L of PCE, 1 mg/L of TCE and 0.4 mg/L of *cis*-DCE were the result of existing Great Lakes soil contamination. The ampules were incubated at 25, 50, 70, and 95 °C, and were destructively sampled after 11.9, 37.4, 75.0, 113.9, and 184.9 days to determine the concentrations of reaction products and rates of degradation as a function of temperature. The resulting data were used to estimate the fraction of PCE mass that could be degraded during in-situ thermal treatment of the Great Lakes site.

4.2.7.1. Thermally-Enhanced PCE Recovery

Heating ampules containing Great Lakes soil and groundwater to greater than 50 °C resulted in increased aqueous-phase concentrations of PCE (Figure 4-37). Results over 185 days of incubation show that aqueous phase PCE concentrations increased with temperature on average by 2.3, 2.7, and 3.2 times at 50, 70, and 95 $^{\circ}$C with respect to 25 °C ampules. However, concentrations were constant over the 185 day incubation period: at 52±1 mg/L in the 25 °C ampules, 121±11 mg/L PCE at 50 °C, 148±10 mg/L at 70 °C, and 171±6 mg/L at 95 °C. The concentration of other compounds present in the Great Lakes soil and groundwater, including *cis*-DCE and TCE, remained constant at 0.4±0.1 mg/L and 1.1±0.4 mg/L, respectively, over the 185 days and at all incubation temperatures. The only exception was a slight increase in TCE concentrations in the ampules incubated at 95 °C from 0.93±0.02 mg/L after 11.9 days to 3.8±0.9 mg/L after 185 days. Given that the aqueous phase solubility limit for PCE is approximately 200 mg/L (Knauss et al., 2000), the PCE concentrations determined in the Great Lakes ampules potentially indicated that non-aqueous phase PCE was present in the Great Lakes soil or that the Great Lakes soil was a strong sorbent for PCE.
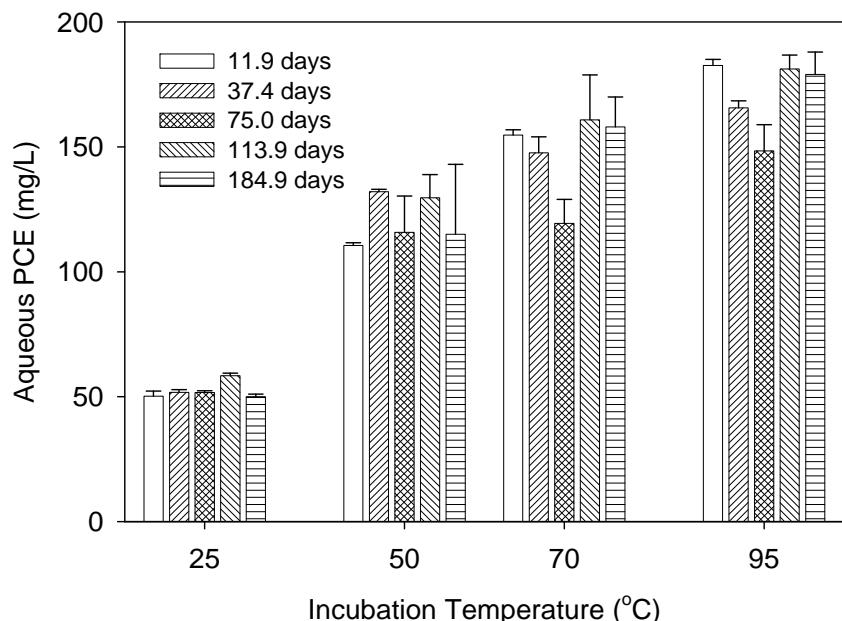
Figure 4-37. Concentration of PCE in aqueous phase of ampules containing Great Lakes soil and groundwater. Each bar represents average of four ampules with the error bar indicating one standard deviation.

While PCE concentrations were increasing, the concentration of PCE in the solid phase of the ampules remained constant at 398±99 mg/kg with incubation temperature and time (Figure 4-38). The mass in the solid phase was expected to decrease to compensate for the mass increase in the aqueous and gas phases, the lack of a decrease suggest that PCE was strongly sorbed by the solid phase. To determine if PCE mass was retained in the Great Lakes solids, sequential methanol extracts of the solid phase were performed for the ampules that had been incubated for 37.5 days (Figure 4-39). While the initial soil PCE concentration was 291±18 mg/kg with one methanol extract, the total soil concentration increased to 641±54 mg/kg after the five methanol extracts. These results show that 54.6% of PCE was strongly sorbed to the Great Lakes soil even after heating for 37.5 days at 95 °C.

The Great Lakes soils are a complex mixture of fine grain minerals, clays, and organic matter. The Great Lakes soil consisted of clay and silt size fractions, with 90% clay (< 0.2 μm) and 10% silt (0.2 to 50 μm) size fractions by weight. The clay minerals present included kaolinite and illite based on Cu X-ray diffraction (XRD) analysis after dithionite-citrate-bicarbonate (DCB) treatment (Jackson, 2005). While the total soil organic content of Great Lakes soils was not determined, extracts from the soil revealed the presence of fulvic acids at 60 mg/L and humic acids at 0.6 mg/L. The metals present included magnesium (1,210 mg/kg) and calcium (3,790 mg/kg) with minor amounts of potassium (12.5 mg/kg) and manganese (23.5 mg/kg). The addition of a strong acid to the Great Lakes soil produced a strong effervescent reaction indicating the presence of carbonates, indicating that the magnesium and calcium were likely part of a carbonate soil fraction. Thus, the sorptive surfaces of Great Lakes soils was likely a combination of carbonates in addition to humic and fulvic organic acids.
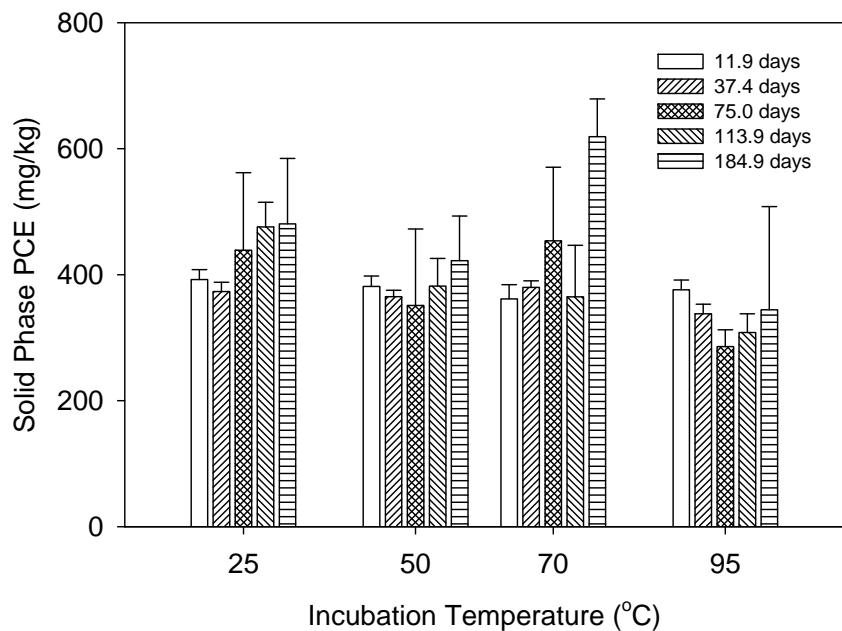
Figure 4-38. Concentration of PCE in solid phase of ampules containing Great Lakes soil and groundwater.
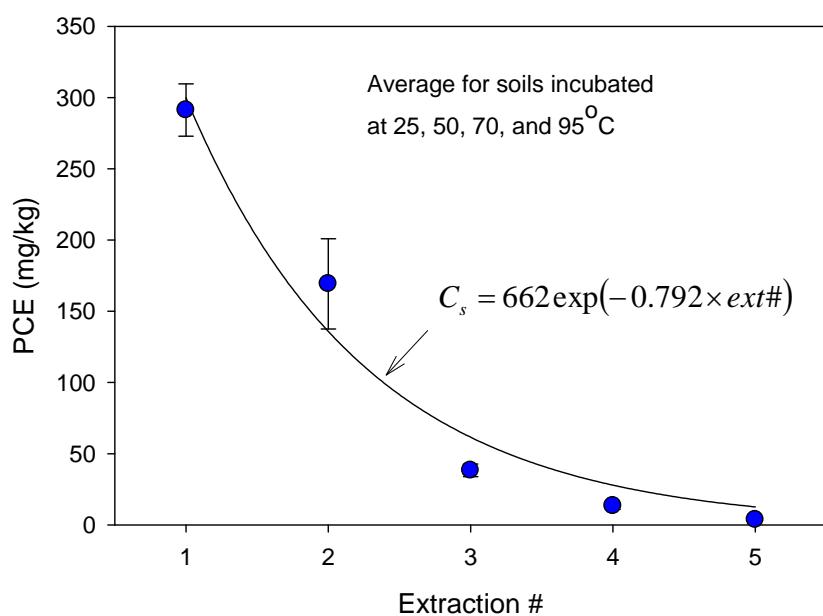
Figure 4-39. Concentration of PCE in solid phase determined by sequential methanol extracts.

In addition to the chlorinated ethenes detected in aqueous and gas phase samples, significant levels of carbon dioxide were found in the gas phase of ampules (Figure 4-40). The carbon dioxide concentrations increased with incubation temperature and time, with the exception of the constant values of 16.4±2% after 75 days at 95 °C. The increase in carbon dioxide and aqueous PCE concentrations with increasing incubation temperature was similarly observed in the Camelot experiments (Section 4.2.5). The correlation between carbon dioxide and PCE concentrations (Figure 4-27) led to the hypothesis that the conversion of carbonate minerals to carbon dioxide gas released sorbed-phase PCE, which then appeared in the ampule aqueous and gas phases.
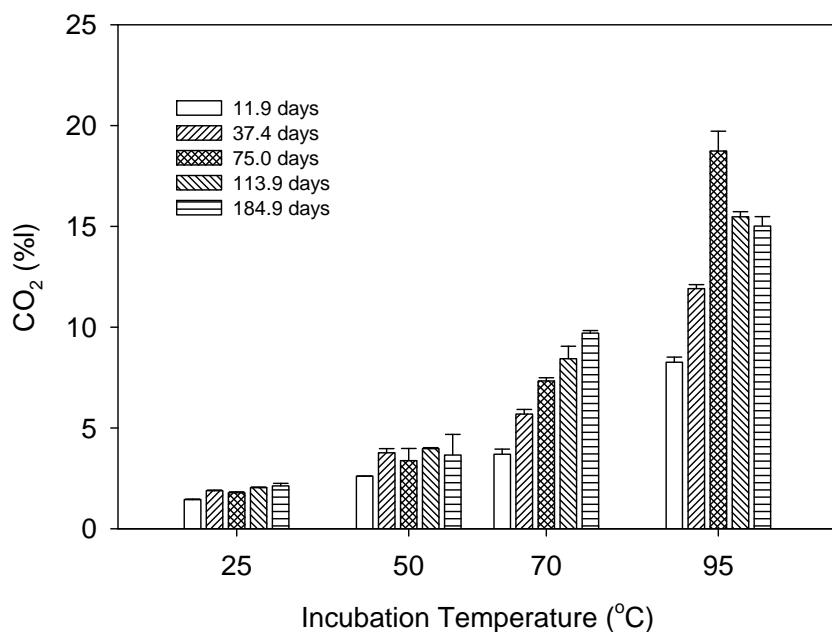
Figure 4-40. Concentration of carbon dioxide in gas phase of ampules containing Great Lakes soil and groundwater.

4.2.7.2. Degradation of Chlorinated Ethenes in Ampules

The observed increase in carbon dioxide may have been due to the degradation of chlorinated ethenes or from the decay of soil components. If the chlorinated ethenes were being degraded then chloride levels would be expected to increase. While chloride levels did increase with temperature (Figure 4-41), they did not increase with time ($p > 0.05$), with the exception of the values obtained after 184 days at 70 and 95 °C. Although the amount by which the chloride increased after 184 days at 70 and 95 °C (41 and 46 μmol, respectively) was similar to that expected based on the increase in carbon dioxide (12 and 17 μmol, respectively). This was the only time that chloride levels increased during the experiment. Also, the ability to detect relatively small changes in chloride levels was hampered by the background chloride levels being at least 10 times greater than the amount of PCE present. In summary, the observed increase in chloride after 184 days at 70 and 95 °C could represent PCE degrading to carbon dioxide or the destruction of soil salts and minerals. While it is possible that PCE, TCE and *cis*-DCE were being degraded during incubation, there was no significant decrease in the amounts of

these chlorinated ethenes during the 185 day incubation period. Since the thermal treatment of the Great Lakes site was completed within a 120 day period, we conclude that the reaction rates were not rapid enough to contribute significantly to mass reductions during electrical resistive heating (ERH) of the Great Lakes site.
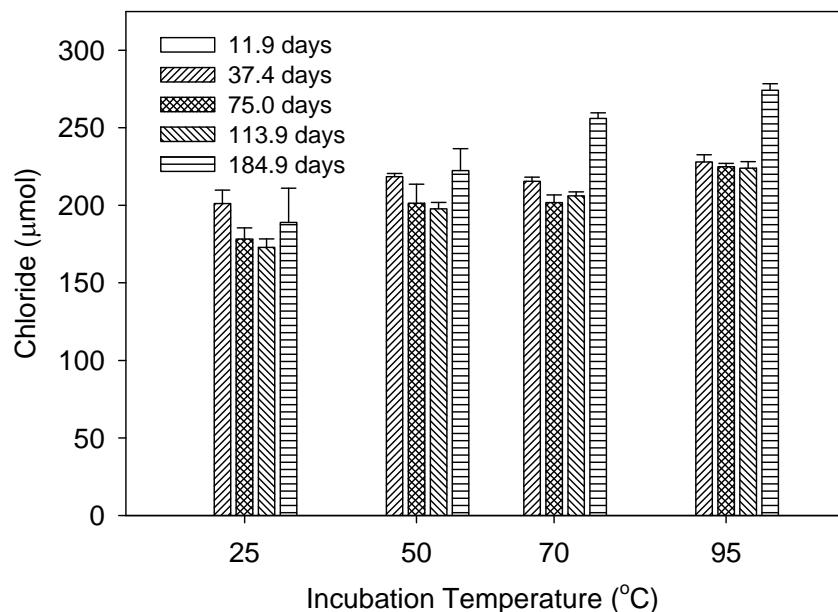
Figure 4-41. Concentration of chloride in the aqueous phase of ampules containing Great Lakes soil and groundwater. Samples from ampules incubated for 11.9 days were not analyzed.

4.2.8. Fate of TCE in Heated Soils from the Pemaco Superfund Site

This study was performed to determine the rate and extent of TCE degradation caused by heating contaminated vadose-zone soil samples collected from the Pemaco Superfund Site located in Maywood, CA. The soil samples, which were collected prior to thermal treatment by electrical resistive heating, were homogenized and humidified with water and gas-phase TCE, loaded into 80 glass-ampules which were then flame sealed. The ampules were incubated at 25, 50, 70, and 95 °C, and were destructively sampled after 13.8, 39.8, 66.9, 125.9, and 189.9 days to determine the concentrations of reaction products and rates of degradation as a function of temperature. The resulting data were used to estimate the fraction of TCE mass that could be degraded during in-situ thermal treatment of the Pemaco site.

4.2.8.1. Variability of TCE Concentrations

The concentrations of TCE in the ampules incubated at 25 °C were variable with time as exhibited by a 9 times increase from day 13.8 to days 39.8 and 66.9 followed by an increase of 2 times at 125.9 and 189.9 days (Figure 4-42). There was also variability in the concentration of TCE among the four ampules at each incubation time, where the standard deviation was greater than the average at days 39.8 and 66.9 (see error bars in Figure 4-42). These results at 25 °C

indicate that the initial TCE distribution in the soil was not uniform. Increasing the incubation temperature had the effect of decreasing the TCE concentration variability as indicated by the decline in RSD from 94% at 25 °C to 79% at 50 °C, 64% at 70 °C and 51% at 95 °C. The decrease in TCE concentration variability also coincided with a decrease in the mass of TCE detected in the soil extracts, which was likely caused by the transfer of TCE from the solid to gas phase. However, since gas phase TCE concentrations were not quantified during this experiment, the supposition that TCE was transferred from the solid to gas phase was not proven.
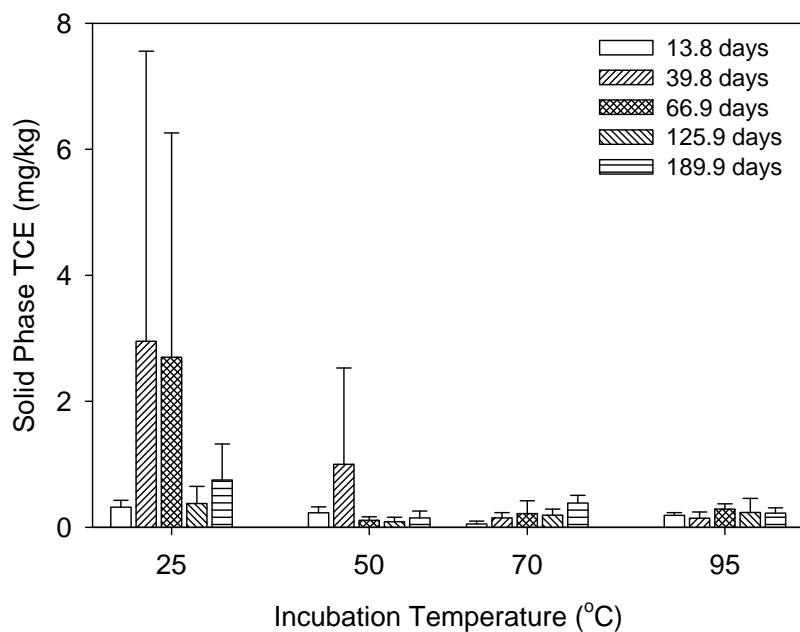
Figure 4-42. Concentration of TCE in aqueous phase extracts of ampules containing Pemaco soil. Each bar represents average of four ampules with the error bar indicating one standard deviation.

4.2.8.2. Degradation of TCE in Heated Pemaco Soil

In addition to TCE, the following products were detected in ampules containing Pemaco soil: carbon monoxide, carbon dioxide, and chloride. The most abundant of these was carbon monoxide where significant concentrations were detected in the ampule gas phase (Figure 4-43). The carbon monoxide concentration had an average value of 16% at 13.8 days and declined with incubation time to 6% after 189.9 days. While carbon monoxide was also observed in the ampules containing Camelot soil and groundwater (Figure 4-28), the levels observed in the Pemaco ampules were approximately 100 times greater. The source of the carbon monoxide was unknown, but most likely originated from either the Pemaco soil, laboratory air, or was potentially introduced during the flame sealing process. Given that these concentrations represent toxic levels, it was unlikely that the carbon monoxide originated from the laboratory air where background concentrations are expected to be less than 5 ppmv or 0.001% (U.S. EPA, 2008). It is unlikely that the carbon monoxide was introduced during the flame sealing process as this procedure was optimized to prevent the introduction of torch flame products by temporarily sealing the ampule with aluminum foil and heating the ampule neck approximately ¾ of an inch

below the ampule opening. Thus, the carbon monoxide observed in ampules containing Pemaco soil and laboratory air was most likely from the Pemaco soil.
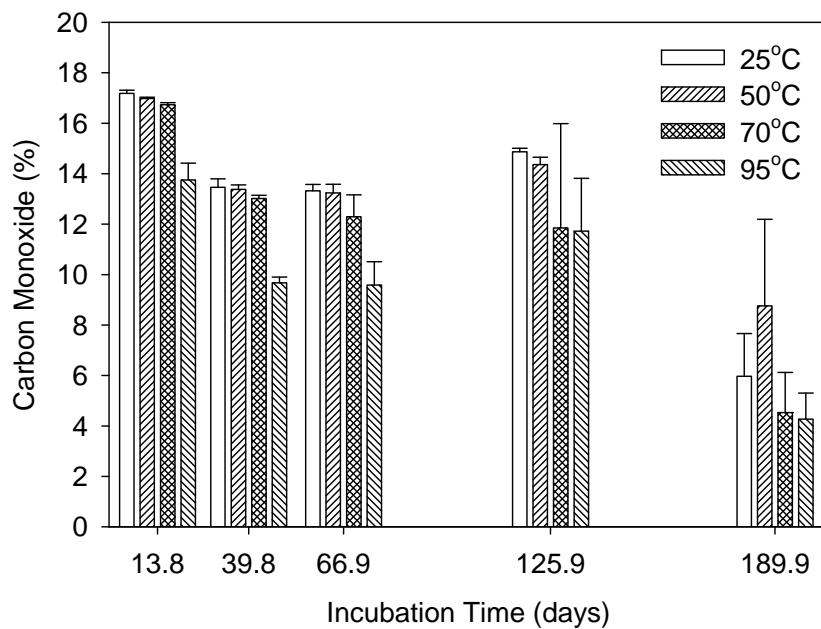
Figure 4-43. Concentration of carbon monoxide in gas phase of ampules containing Pemaco soil. Each bar represents average of four ampules with the error bar indicating one standard deviation.

While there was slightly less carbon monoxide in the ampules incubated at 95 °C as compared to the concentrations at 25, 50, and 70 °C, the trend of decreasing concentrations with incubation time was independent of incubation temperature. This result suggests that carbon monoxide was being consumed by some fraction of the Pemaco soil during incubation. While carbon monoxide was being consumed, the concentration of carbon dioxide was increasing with incubation time up to 125.9 days (Figure 4-44). The concentration increase was greatest in the ampules incubated at 95 °C, and in general increased with incubation temperature. This trend of increasing carbon dioxide concentrations did not continue through 189.9 days of incubation however. While the exact cause of the decline in carbon dioxide between 125.9 and 189.9 days remains unknown, it does suggest that, like carbon monoxide, some fraction of the Pemaco soil was consuming the carbon dioxide at 70 and 95 °C after 125.9 days.
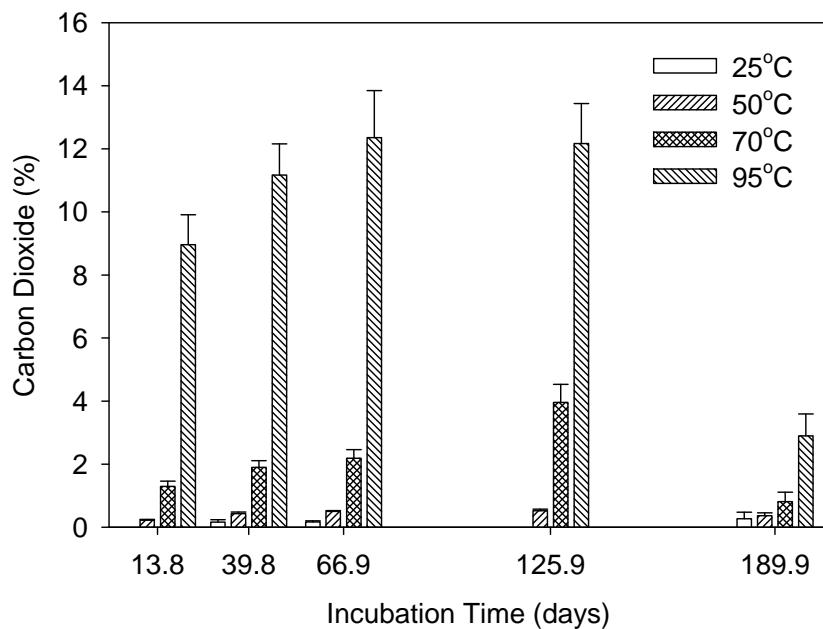
Figure 4-44. Concentration of carbon dioxide in gas phase of ampules containing Pemaco soil. Each bar represents average of four ampules with the error bar indicating one standard deviation.

Along with carbon monoxide and dioxide in the gas phase, chloride was detected in the aqueous soil extracts (Figure 4-45). The chloride concentrations were constant regardless of incubation temperature or time indicating, and relatively low compared to the concentrations determined in the Camelot (Section 4.2.5) or Great Lakes (Section 4.2.7) experiments. However, even these low chloride concentrations were approximately 100 times greater than the concentrations of TCE meaning that any small change in chloride concentration due to the dechlorination of TCE would be difficult to distinguish from the background chloride. Thus, while it cannot be ruled out that TCE was being dechlorinated during incubation of the Pemaco ampules, the lack of increase in chloride levels suggests that dechlorination of TCE was not occurring in the ampules at temperatures below 95 °C and for incubation times of less than 189.9 days.
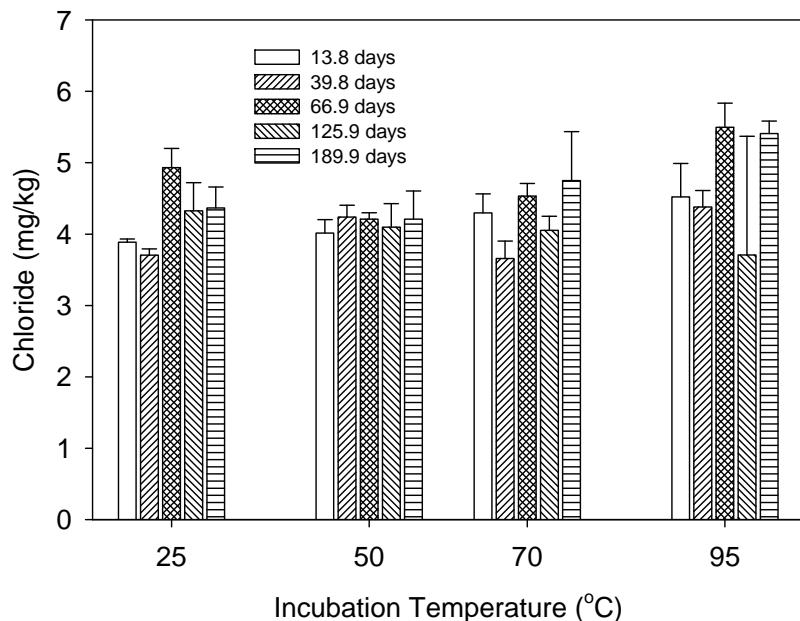
Figure 4-45. Concentration of chloride in aqueous phase extracts of ampules containing Pemaco soil. Each bar represents average of four ampules with the error bar indicating one standard deviation.

4.2.9. Reactivity of PCE and TCE with Persulfate

The ampule incubation experiments completed using field contaminated samples, as described in the previous sections, demonstrated that TCE and PCE may undergo abiotic degradation at elevated temperatures; however, the observed rates were relatively slow. Thus, chlorinated solvent recovery during thermal treatment is likely to be dominated by enhanced mass transfer from the solid and liquid phases, while in-situ transformation processes provide only minimal contributions to PCE destruction. Additional findings from ER-1419 experiments show that while the rate of PCE desorption from field contaminated soils was accelerated with increasing soil temperature, the contaminants remained in soils even after heating at 95 °C for up to 185 days. Thus, even after removal of condensed phase (NAPL) chlorinated ethenes, a substantial fraction of contaminant mass can remain associated with the solid phase, particularly in low-permeability soils (e.g., Camelot, Great Lakes). This slowly desorbing fraction of contaminant may require prolonged heating to achieve remediation goals, and could result in the rebound of groundwater concentrations once thermal treatment ceases. Based on these observations, the addition of reactive amendments capable of promoting in-situ contaminant destruction could (a) reduce the duration and temperature of thermal treatment, and (b) serve as a polishing step to treat slowly desorbing contaminants during site cool down or zones of persistent high contamination (i.e., hot spots) during active thermal treatment.

Given the relatively minor enhancements in chlorinated solvent degradation observed at elevated temperatures, we completed experiments with sodium persulfe that can be used to facilitate or enhance reactivity during thermal treatment. A series of experiments were undertaken to investigate the ability sodium persulfate to enhance PCE degradation in aqueous

102

solutions and in the presence of solids. Kinetic batch experiments were completed using 8 mL glass vials sealed with screw-top caps, where persulfate was added to 6 vials and another other 6 vials served as persulfate-free controls. After equilibrating in a recalculation water bath for 5 minutes to reach the experimental temperature, the vials were removed and then a 32 mM sodium persulfate solution with pH 7.0 phosphate buffer was added 6 of the vials and an equivalent volume of DI water was added tot he other 6 vials and the vials were then returned to the water bath. Pairs of vials were collected at regular intervals and destructively sampled to determine the concentration of PCE, chloride, and sulfate with increasing reaction times.

4.2.9.1. Reactivity of PCE with Persulfate in Water Alone

The rate of PCE oxidation by phosphate-buffered sodium persulfate, along with the rate of chloride and sulfate production, were determined in DI-water containing reactors at temperatures of 31.1, 40, 50, 60, and 70 °C. These water only trials were completed to demonstrate that the experimental protocol would yield accurate kinetic data before completing experiments with solids present. PCE disappearance and chloride appearance during the water only experiments were best described using a first-order kinetic model for reaction times that ranged from 2.4 half-lives at 40°C up to 5.5 half-lives at 60 °C (Figure 4-46). No downward curvature at the late time points, as reported by Waldemer et al. (2007), was observed in these experiments.
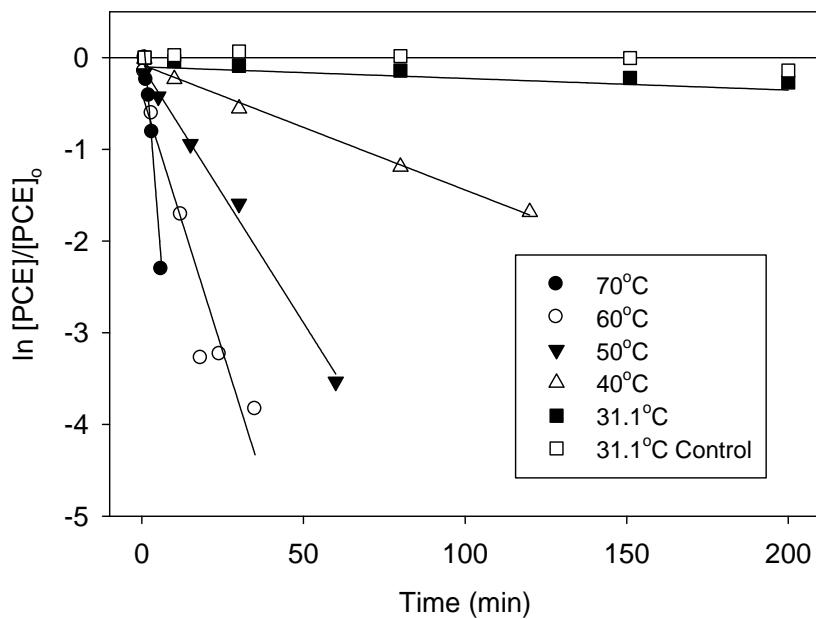
Figure 4-46. First-order oxidation of PCE by phosphate-buffered sodium persulfate at five temperatures.

Figure 4-47 shows the results from the experiment completed at 50 °C showing the first-order fit for PCE disappearance and chloride appearance, along with a constant PCE concentration of 105 ± 9 μM in the control reactors. The amount of chloride formed at each time

point was on average within 15% of the amount of PCE oxidized, assuming complete PCE dechlorination with 4 moles of chloride being produced for each mole of PCE oxidized. The good chloride to PCE mass balance and lack of chlorinated intermediates, such as *cis*-DCE and TCE above the 0.1 mg/L detection limit, led to the conclusion that PCE was completely dechlorinated by phosphate-buffered sodium persulfate in DI-water containing reactors at 50 °C. The stoichiometry for the oxidation of PCE by sodium persulfate at 50 °C, based on the ratio of products after 99% of the initial PCE mass was oxidized, was described by the following equation:

$$2C_2Cl_4 + Na_2S_2O_8 + 8H_2O \xrightarrow{\Delta} 2SO_4^{2-} + 8Cl^- + 4CO_2 + 4H^+ + 6H_2 + 2Na^+ \qquad (4.9)$$
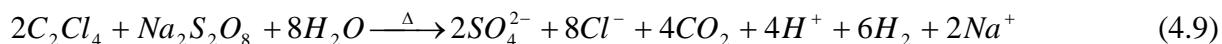
Equation 4.6 assumes that carbon dioxide is the principle PCE oxidation product as no organic acids were detected above the 10 μM detection limit. Thus, there was a 2:1 stoichiometric ratio of PCE oxidized to persulfate consumed in these water-only experiments. These conclusions also apply to the experiments completed at 31.1, 40, 60, and 70 °C where the amount of chloride formed to PCE oxidized was on average within 11, 10, 16, and 18%, respectively.
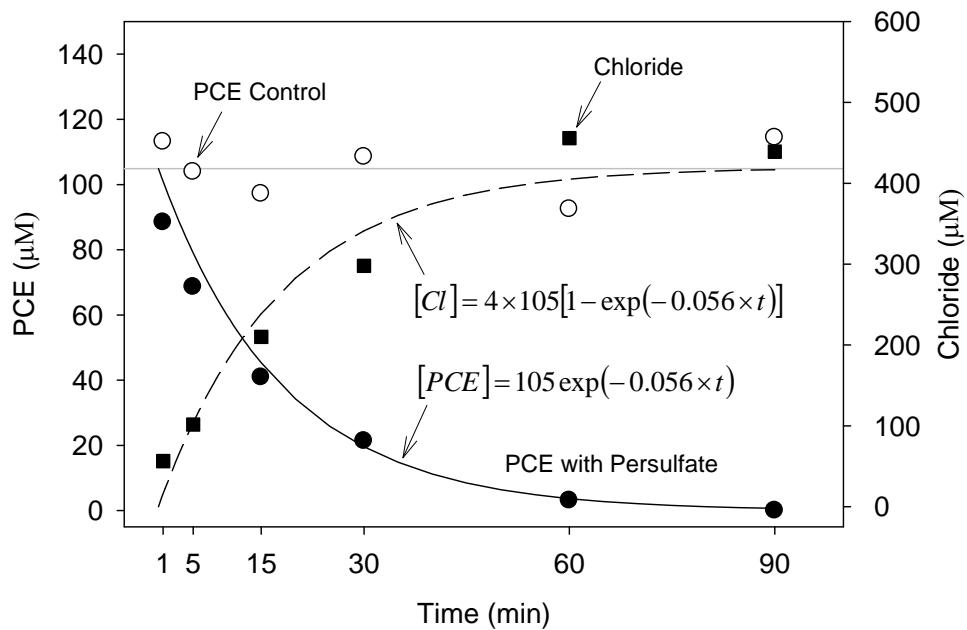
Figure 4-47. Concentration of PCE and chloride in vials containing PCE and phosphate-buffered persulfate heated to 50 °C. Controls were identically prepared reactors with the exception of persulfate.

The Arrehnius rate parameters were calculated using the rate of PCE oxidation determined for each temperature excluding the rate determined for the 31.1 °C experiment. Results from the 31.1 °C experiment were excluded because the rate did not fit with those determined for the other temperatures, potentially indicating a difference in reaction mechanism

at temperatures above 31.1 °C. The apparent activation energy (E_a) was determined to be 101.8±4.7 kJ/mol, which was within 1% of the value reported by Waldemer et al. ([2007]), and the natural log of the pre-exponential factor (ln A) was 30.8 ± 1.7 1/s. The pre-exponential factor was ca. 6 times greater than the value reported by Waldemer et al. (2007) indicating that the rate of PCE oxidation determined in these experiments was on average 4.4 times faster for all temperatures except at 31.1 °C, where the rates were within 18% of each other. For example, the rate of PCE oxidation at 50 °C had a first order half-life of 12.4 ± 0.6 min and PCE was below the detection limit of 0.1 mg/L after 90 min. This rate was 5 times faster than the rate calculated from the data reported by Waldemer et al. (2007) and the extent of PCE degradation was also five times greater. The slower rates were determined without using a pH buffer, whereas the faster rates reported herein were obtained using a pH 7.0 phosphate buffer. Decreases in the rate of oxidation with unbuffered persulfate solutions was previously reported by Goulden and Anthony (1978) and is thought to be due to a lowering of solution pH caused by the production of hydrogen ions during persulfate consumption (see Eg. 4.6). A decrease in the rate of TCE degradation with decrease in pH was also reported by Liang et al. (2007) for persulfate oxidation by iron activation at ambient temperature (i.e., 25 °C). Use of a buffer for these experiments is justified in that subsurface environments have significant buffer capacity where the presence of phosphates and carbonates are common. The experimental protocols were judged to yield accurate kinetic data based on these water-only results.

4.2.9.2. Persulfate Reactivity with PCE in Water and Soil

After completing the water-only experiments over the range of temperatures from 31.1 to 70 °C, a series of experiments were completed at 50 °C with solids containing reactors to determine the effect of solids on the rate and extent of PCE oxidation. The presence of these solids either had no effect on the rate and extent of PCE oxidation by heat activated persulfate at 50 °C, or prevented persulfate from oxidizing PCE (Table 4-5). With borosilicate glass beads present, the first-order half-life of PCE was 12.4 min and was not significantly different ($p < 0.05$) than the half-life of 12.4 min obtained in the water-only experiment. After 80 minutes (9.6 half-life), 93% of the PCE was oxidized and the amount of chloride was within 2.5% of the amount of PCE oxidized. The stoichiometric ratio of PCE oxidized to persulfate consumed decreased from 2:1 in water only experiments to ca. 2:3 with the borosilicate glass beads present indicating that more persulfate was consumed with the glass beads present. Thus, borosilicate glass beads increased the amount of persulfate consumed but did not affect the rate or extent of PCE oxidation. The presence of Fort Lewis soil and lime glass beads caused the rate of PCE oxidation to decrease, where the first-order rate coefficient was approximately two times lower than that determined in water only experiments, while the extent of PCE oxidation after 80 min was not significantly affected (i.e., 93 and 92%). The chloride to PCE mass balance was within 4% for the lime glass beads, but was more variable with the Fort Lewis soil where only half of the chloride expected was present after 80 min which then was within 8% after 120 min. The PCE to persulfate ratio was ca. 3:2 for the lime glass beads and ca. 1:5 with Fort Lewis soil indicating that more persulfate was consumed with the field soil than with the lime glass beads.

Table 4-5. Half-life, rate, and extent of PCE oxidation.

Reactor Content	Half-Life (min)	Rate (1/hr)[1]	Extent of Reaction at 80 min (C/Co %)		Ratio PCE:Persulfate
			PCE	Persulfate	
Water Only	12.4	-3.36±0.15[2]	99	1.3	2.0
Borosilicate Glass Beads	12.4	-3.34±0.20	93	3.9	0.7
Fort Lewis	22.1	-1.82±0.13	92	6.4	0.2
Lime Glass Beads	23.2	-1.80±0.32	93	1.9	1.4
Illite-Smectite	58.0	-0.72±0.09	63	0.9	0.3
F-70 Sand	83.8	-0.50±0.09	52	1.7	0.2
Montmorillonite	134.2	-0.31±0.10	32	1.7	0.5
20-30 Mesh Sand	187.3	-0.22±0.14	22	2.6	0.4
Kaolinite	312.6	-0.13±0.10	17	4.9	0.1
Great Lakes	NA[3]	No reaction	NA	0.3	NA
Appling	NA	No reaction	NA	7.0	NA

[1]Rate determined using all data through 80 min. [2]Standard error. [3]not applicable

Further decreases in the rate and extent of PCE oxidation were observed for the reference clays and sands where the first-order rate coefficients were from 5 to 26 times lower for these reference solids as compared to the water only experiment. While there was a decrease in the rate and extent of PCE oxidation, the amount of persulfate consumed varied from a low of 0.9% for the Illite-Smectite to 4.9% for the kaolinite. The decrease in PCE oxidation rate coefficient for the reference clays and sands was linearly related to the increase in amount of persulfate consumed with a correlation coefficient (r^2) of 0.96. This result suggests that persulfate was being consumed by soil minerals in preference to PCE. Results for these low organic carbon (i.e., > 0.001%) reference materials demonstrate that the mineral fractions associated with the solid surfaces can affect persulfate oxidation of PCE. While PCE was oxidized by persulfate in reactors with the field soil from Fort Lewis, there was little decrease in PCE content in the reactors containing field soil from Great Lakes and Appling. Appling soils exhibited the greatest persulfate consumption of the solids while Great Lakes had the least, further complicating the interpretation of these experimental results.

4.2.9.3. Sequential Persulfate Treatment

Although there was a substantial fraction of persulfate remaining (i.e., > 93%, Table 4-5) after 80 min at 50 °C in all solids containing reactors, the reactors were sequentially treated with persulfate solution to determine if the total oxidant demand could be satisfied and thereby improve the rate of PCE oxidation to that of the borosilicate glass bead reactors. The total soil oxidant demand is thought to comprise the sum of reduced subsurface minerals and natural

organic matter, in addition to organic contaminants (Haselow et al., 2003). Thus, the decrease in the rate and extent of PCE oxidation observed in the solids containing reactors may have been due to competition for the persulfate oxidant by oxidizable components other than PCE. As shown in Figure 4-48, the extent of PCE oxidation after 80 min at 50 °C increased for reactors containing soda lime glass beads, reference sands (F-70 and 20-30 mesh Ottawa sands), and for two of the reference clays (kaolinite and montmorillionite). However, no increase was observed for illite-smectite or the field soils (Appling, Fort Lewis, and Great Lakes).

These results suggest that the oxidant demand associated with soil mineral fraction of the reference sands and clays was more easily met than the demand associated with the Appling and Great Lakes soils. For the reference clays and sands, where the rate and extent of PCE oxidation increased with sequential treatment, there was also an increase in the amount of persulfate consumed after 80 min at 50 °C (Figure 4-49). This result is consistent with the hypothesis that the reduced mineral fraction of the total soil oxidant demand competes with PCE oxidation, and that once these mineral fractions are oxidized, the radicals formed by heat activated persulfate decomposition would be available to react with persulfate and PCE. Thus, increases in the amount of persulfate decomposed and extent of PCE oxidized both increased with sequential treatment. This also demonstrates that the soil oxidant demand must be satisfied before PCE oxidation can take place.
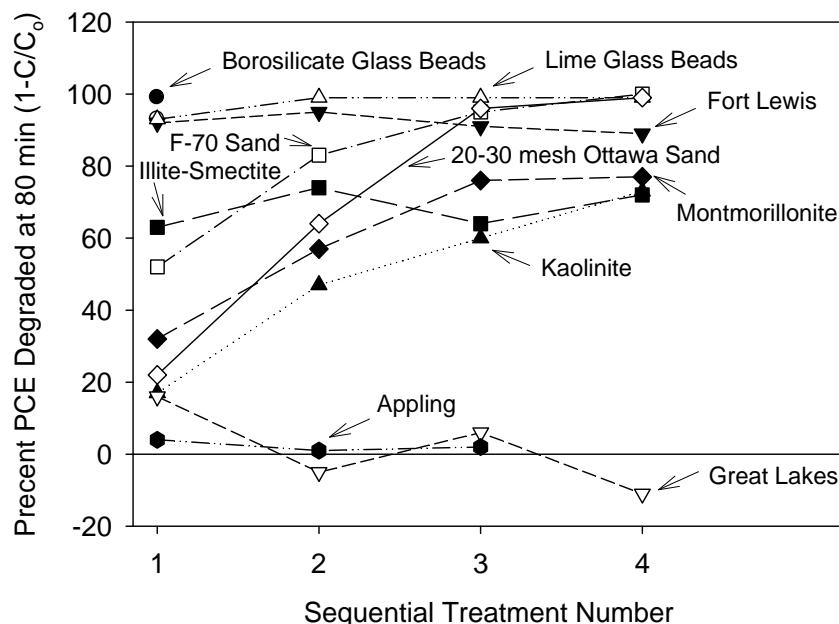
Figure 4-48. Extend of PCE oxidation with sequential persulfate treatment after 80 min at 50 °C. Only the aqueous phase was replaced between subsequent treatments.

For the field soils, the rate and extent of PCE oxidation was unchanged by repeated application of persulfate. Given that these field soils contain natural organic matter (NOM) in addition to minerals, it was hypothesized that the NOM was preventing improvements in PCE

oxidation. To test this hypothesis experiments were performed after treating the Great Lakes and Appling soils with a 30% hydrogen peroxide solution to remove all oxidizable soil fractions (Jackson, 2005). Even after repeated hydrogen peroxide treatment, the rate and extent of PCE oxidation was not improved. Thus, there remained some soil fraction that was interfering with the persulfate oxidation of PCE which could not be removed by repeated hydrogen peroxide or persulfate application.
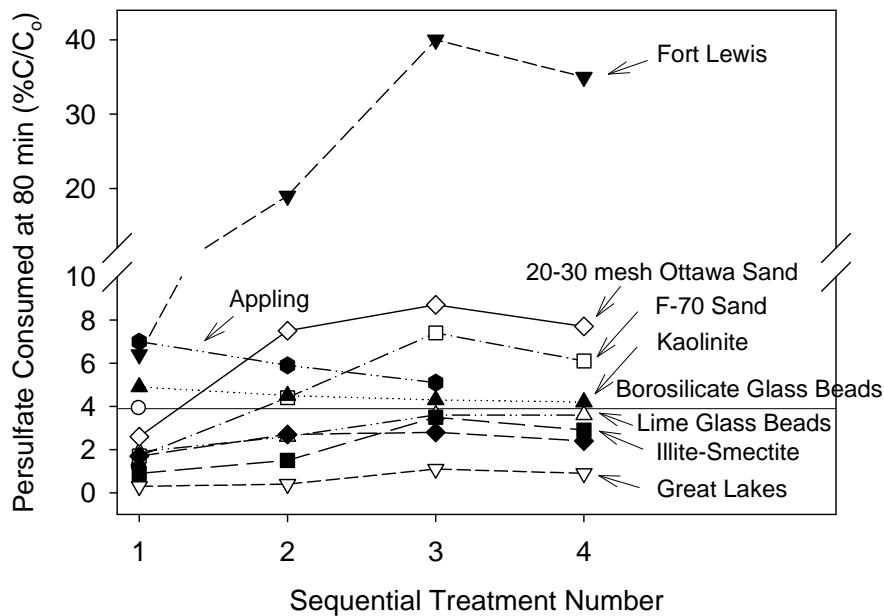
Figure 4-49. Amounts of persulfate consumed by each solid phase.

4.2.10. Reactivity of TCE in a Heated Quartz Tube

A high-temperature flow-through experiment was designed to simulate soil vapor extraction of TCE vapors, which occurs during thermal treatment of subsurface environments contaminated with TCE. Experiments were completed in a 0.5 L quartz-tube that was used to study short residence time (<10 minutes) conditions which occur during recovery of contaminated vapor, an integral part of the thermal treatment process. The procedures and methods for this work were based on those documented in Costanza et al. (2007d), which established that significant TCE degradation (i.e., > 5%) only occurred in the experimental system at temperatures great than 420 °C. The principle TCE degradation products were CO and CO_2, however, only when oxygen was present. Otherwise, TCE degraded into chlorinated hydrocarbons that contained four and six carbon atoms with greater than five chlorine atoms per molecule (i.e., perchlorinated hydrocarbons).

The experiments completed herein were at temperatures of 120, 240, 380, and 420 °C as a function of quartz tube water vapor content. The vapor content was controlled by heating the DI water containing mini-bubbler, used to humidify the influent carrier gas, and is reported here

as inlet temperature. Two additional experiments were completed at 800 $^{\circ}$C, with dry air, to determine the products formed at the maximum temperature reportedly used during in situ thermal conductive heating.

4.2.10.1. Recovery of TCE from Heated Quartz Tube Reactor

The amount of TCE recovered after each temperature trail is provided in Table 4-6 and shown in Figure 4-50. The average recovery of TCE for the experiments completed at 120 °C was 96.4 ± 3.0% demonstrating that the cold trap proved to be good at collecting TCE after passing through the heated quartz tube. The one exception was the relatively low (93.1%) TCE recovery when the inlet temperature was at 100 $^{\circ}$C, which represents the maximum water vapor content. In this case, the toluene in the 40 mL trap was pulled into the cold-trap during a carrier gas flow interruption resulting in TCE bypassing the trap, this was reflected in the low TCE recovery for the experiment with inlet temperature of 100°C. The average recovery of TCE in the 240 °C experiments was 99.5±1.9% and then declined to 84.8±4.6% at 380°C and to 67.9±13.9% at 420 °C.

Table 4-6. TCE recovery with air as the carrier gas (Recovery = TCE in cold-trap ÷ TCE injected × 100%).

Inlet Temperature (^{o}C)	Quartz Tube Temperature (°C)			
	120	240	380	420
dry	Nd	98.3±1.8	nd	nd
22	98.7±2.2[a]	97.9±0.6	81.7±2.3	61.1±1.0
60	97.5±2.2	99.7±2.2	82.6±1.1	58.7±0.3
100	93.1±1.8	102.2±1.9	91.1±1.6	84.0±1.1
Average ± Standard Deviation	96.4±3.0[b]	99.5±1.9	84.8±4.6	67.9±13.9

nd – not determined
[a]Analytical variability: standard deviation based on analysis of three samples.
[b]Experimental variability: standard deviation of TCE recovery for differing water contents.
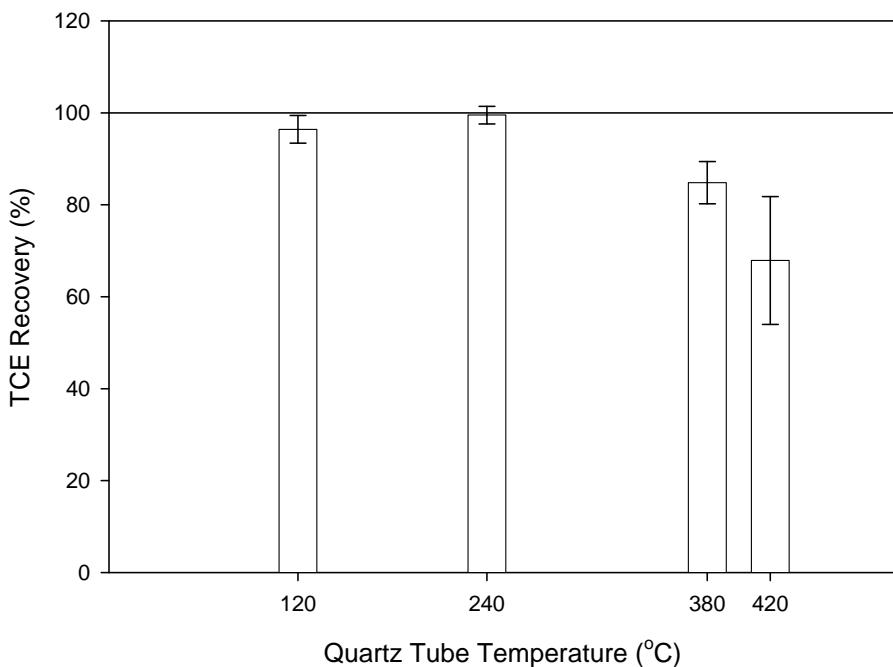
Figure 4-50. Recovery of TCE as a function of quartz tube temperature for the high-temperature flow-through experiment.

4.2.10.2. Recovery of TCE Degradation Products from Heated Quartz Tube

While no compounds other than TCE were found above the detection limit of 1 mg/L in the effluent from the 120 and 240 °C experiments, there were a variety of products detected in the effluent from the 380 and 420 °C experiments. Table 4-7 contains the compounds detected in experiments completed at 380 °C with air as the carrier gas. The predominant TCE degradation products on a carbon basis included CO, phosgene, CO_2, and hexachloroethane. The overall carbon balance was greater than 90% for each experiment completed at 380 °C demonstrating that the principle degradation products were accounted for. The amount of TCE recovered increased from 81.7 to 90.1% with an increase in quartz tube water content from inlet temperature of 22 to 100 °C. The increase in TCE recovery was matched by a decrease in the amount of TCE degradation products formed: CO decreased from 6.1 to 2.7%, phosgene from 4.0 to 0.2%, and hexachloroethane from 0.8 to 0.3%, while CO_2 levels increased from 1.2 to 1.6%.

The amount TCE degraded increased at 420 °C and was accounted for by increases in the amounts of CO, phosgene, CO_2, hexachloroethane, PCE and CCl_4 (Table 4-8). The effect of increasing the water content at 420 °C resulted in an increase in TCE recovery and a decrease in the amount of degradation products formed. Phosgene decreased from 8.7 to 0.5%, hexachloroethane from 4.2 to 0.5%, and PCE from 2.8 to 0.5%.

110

Table 4-7. Carbon balance for the 380 ^{o}C experiments with air as the carrier gas.

Compound	Inlet Temperature (°C) or Water Content		
	22	60	100
TCE	81.7±2.3[a]	82.6±1.1	90.1±1.6
CO	6.1	5.7	2.7
Phosgene	4.0±0.1	0.6±0.0	0.2±0.0
CO_2	1.2	1.4	1.6
Hexachloroethane	0.8±0.2	1.0±0.0	0.3±0.0
PCE	0.6±0.1	0.7±0.0	0.1±0.0
Hexachlorocyclopropane	0.4±0.1	0.5±0.1	0.2±0.0
Chloroform	0.1±0.0	0.1±0.0	0.1±0.0
CCl_4	0.1±0.0	0.1±0.0	0.0±0.0
Dichloroacetate	0.02	*Lost*	0.00
Trichloroacetate	0.07	*Lost*	0.00
Net Recovery (%)	95.1±2.6	92.8±1.2	95.2±1.7

Lost – Sample lost during dervatization process due to vial failure.
[a]Analytical variability: standard deviation based on analysis of three samples.

Table 4-8. Carbon balance for the 420 ^{o}C experiments with air as the carrier gas.

Compound	Inlet Temperature (^{o}C) or Water Content		
	22	60	100
TCE	61.1±1.0[a]	58.7±0.3	84.0±1.1
CO	14.3	15.7	7.7
Phosgene	8.7±0.4	1.9±0.0	0.5±0.1
CO_2	2.6	6.0	4.0
Hexachloroethane	4.2±0.1	3.8±0.1	0.5±0.0
PCE	2.8±0.1	2.8±0.1	0.5±0.0
CCl_4	1.0±0.1	0.7±0.0	0.1±0.0
Hexachlorocyclopropane	0.6±0.0	0.5±0.1	0.2±0.0
Pentachlorobutadiene	0.4±0.0	0.4±0.0	0.1±0.0
Hexachlorobutadiene	0.4±0.0	0.4±0.0	0.1±0.0
Hexachlorobutane	0.4±0.0	0.3±0.0	0.1±0.0
Hexachlorobenzene	0.3±0.0	0.2±0.0	0.1±0.0
Chloroform	0.1±0.0	0.1±0.0	0.2±0.0
Dichloroacetate	0.02	0.04	0.00
Trichloroacetate	0.22	0.02	0.00
Net Recovery (%)	97.1±1.9	91.7±0.6	98.1±1.2

[a]Analytical variability: standard deviation based on analysis of three samples.

111

4.2.10.3 High Temperature Quartz Tube Experiments

Two additional experiments were completed with the quartz tube heated to 800 $^{\circ}$C using dry air as the carrier gas. The carrier gas was not humidified as the stability of the quartz tube heated to this temperature was a concern. The initial experiment used a toluene trap in series after the cold trap; the same configuration used for the previous experiments described in this section. The only compounds detected after passing TCE through the quartz tube heated to 800 $^{\circ}$C were CO_2, CO, and phosgene. No other compounds were found in the cold-trap, the toluene trap, or in the toluene rinse of the quartz tube. Unfortunately, these compounds only accounted for 77.3% of the carbon introduced as TCE (Table 4-9).

Table 4-9. Carbon balance for the 800 $^{\circ}$C experiments with air as the carrier gas.

Compound	Toluene Trap	Water Trap
TCE	Not detected	Not detected
CO_2	42.0	49.8
CO	34.6	33.7
Phosgene	0.6	0.1
Net Recovery (%)	77.3	83.6

A second 800 $^{\circ}$C experiment was completed using a 40 mL vial filled with DI water in place of the toluene trap. The water trap was added in attempt to account for the chlorine liberated during TCE degradation. While the amount of CO_2 increased from 42.0 to 49.8% and the amount of phosgene decreased from 0.6 to 0.1% when the toluene trap was replaced by a water trap, the carbon balance was still not complete with 16.4% of the carbon unaccounted for. The pH of the water in the trap decreased from 7.0 to 1.20, which was expected to indicate that HCl had formed as a product of TCE degradation. However, chloride (Cl^-) was not detected in the water trap or the quartz tube rinse above the colorimetric analysis method detection limit of 0.1 mg/L. The water in the trap had a characteristic order that was similar to that described for Cl_2 gas. A 1 mL gas sample collected from the Tedlar$^{®}$ bag was injected into a GC-MS and in addition to a chromatogram peak identified as phosgene; there was another elution peak that had a mass spectrum base peak with m/z of 70, which is characteristic for the Cl_2 molecule. The location of the missing carbon is unknown at this time.

4.2.10.4. Implications for Thermal Treatment

The maximum temperature anticipated during electrical resistive heating (ERH) and steam flushing is less than 240 °C (Costanza et al., 2007d). Based on the results of these experiments, no significant degradation of TCE is expected to occur during the short residence-time vapor recovery at ERH or steam flushing treatment sites.

There are three broad temperature regions that develop during thermal conductive heating including a 100 to 250 °C region located between heater wells, a 500 to 700 °C region for soil within a one-foot radius of heater wells, and the 745 to 900 °C region located within heater wells. The experiments performed at 120 and 240 °C suggest that TCE would not be expected to degrade at these temperatures. As gas phase TCE flowed through soil heated to greater than 380

°C, the potential to degrade TCE would be anticipated based on the results obtained from experiments completed at 380 and 420 °C.

In subsurface environments where oxygen is present, TCE is anticipated to readily degrade at temperatures in excess of 380°C based on results from this experiment where the quartz tube was heated to 380 and 420 ^{o}C with air as the carrier gas. The average amount of TCE recovered after passing through the quartz tube heated to 380°C was 84.8% and decreased to 67.9% with the quartz tube heated to 420 °C. The primary TCE degradation products at 380°C and 420 ^{o}C with oxygen present included CO, phosgene, and CO_2 (Table 4-7 and 4-8). These compounds would be expected to remain in the gas phase once formed and travel to subsurface volumes that were at lower pressure. A properly designed and operated vapor extraction system would be expected to recover these compounds.

At 420 °C, chlorinated compounds in addition to phosgene were found after passing TCE through the quartz tube with air as the carrier gas. Hexachloroethane, PCE, and CCl_4 were present at greater than 1% of the amount of TCE introduced into the quartz tube (Table 4-8). A general guide for removing a compound from soil via ex situ thermal treatment is that the soil must be heated to at least the boiling point temperature of the compound (Mechati et al., 2004). The boiling point temperature for hexachloroethane, PCE, and CCl_4 are all less than 420 ^{o}C (Table 3-6), therefore, these compounds would be expected to remain in the gas phase once formed. Assuming that the vapor recovery systems used during thermal remediation was capable of capturing these TCE degradation products, then they would be anticipated to travel toward the in situ well heated to 800 °C.

The experiments completed by heating the quartz tube to 800 °C with air as the carrier gas represented an ideal system where the compounds formed during TCE degradation were expected to be recovered based on the net carbon recovery of greater than 91.7% for experiments completed at 380 and 420 °C. In the 800°C experiment, TCE passed from room temperature (25 °C) through an approximate 10 inch long region of increasing temperatures before entering the 800 °C zone within the quartz tube, then through a decreasing temperature region, and ending up in the cold trap at -78 °C. TCE was degraded to CO, CO_2, and a small amount (<1% carbon basis) of phosgene (Table 4-9) and no hexachloroethane, PCE, or CCl_4 was detected in the experiments completed at 800°C with air as the carrier gas. Despite the fact that the compounds found in the lower temperature experiments (e.g., hexachloroethane) probably formed during the 800°C experiment, they were not found in the cold trap employed to capture TCE degradation products or in the toluene rinse of the quartz tube. Thus, these compounds were degraded in the quartz tube heated to 800 °C. However, the amount of missing carbon in the well controlled 800 °C experiments (~17%) makes the prospect of recovering all the TCE degradation products during thermal conductive heating is doubtful.

4.3. MICROBIAL REDUCTIVE DECHLORINATION AT ELEVATED TEMPERTURE

4.3.1 Spore Formation in Response to Heating

A variety of bacteria including *Dehalobacter*, *Desulfitobacterium*, *Desulfuromonas*, *Geobacter*, and *Sulfurospirillum* spp. reductively dechlorinate the groundwater contaminants tetrachloroethene (PCE) and trichloroethene (TCE) to *cis*-1,2-dichloroethene (*cis*-DCE) (Gerritse et al., 1996; Holliger et al., 1998; Scholz-Muramatsu et al., 1995; Sung et al., 2006a; Sung et al., 2003). A number of PCE-dechlorinating *Desulfitobacterium* strains as well as *Clostridium bifermentans* strain DPH-1 (Chang et al., 2000; Villemur et al., 2006) are also capable of spore formation. Spore-forming PCE dechlorinators may play relevant roles for initiating dechlorination following exposure to unfavorable conditions during physical-chemical remediation, including thermal treatment of PCE and/or TCE source zones. The goal of this study was to explore the spore-formation capability of the known PCE-to-*cis*-DCE-dechlorinating *Clostridium* isolate.

4.3.1.1 Resolution of Culture DPH-1 into Two Populations

A reportedly pure culture of *Clostridium bifermentans* DPH-1 was obtained from K. Takamizawa. The culture reduced PCE to stoichiometric amounts of *cis*-DCE in 10 to 20 days, and this activity was stable upon repeated transfers. Prior to spore formation assays, to ensure culture purity, culture fluid was spread on Luria Bertani (LB) agar plates inside an anoxic chamber. Uniform colonies formed on plates within 1 week of incubation, but, unexpectedly, when cells from isolated colonies were transferred to liquid medium, PCE dechlorination activity was not recovered although visible growth occurred within 1 day (Figure 4-51).

In order to isolate the organism responsible for PCE dechlorination, two sequential dilution-to-extinction series were performed. In the first dilution-to-extinction series, dechlorination of PCE to *cis*-DCE occurred in the 10^{-10} dilution vial. This vial served as the source for the second dilution-to-extinction series. In the second dilution-to-extinction series, dechlorination activity occurred in the 10^{-9} dilution vial. When aliquots from this culture were spread onto LB agar plates, no colonies formed. Microscopic analysis corroborated the presence of two distinct organisms in the original DPH-1 culture. Slender rods were observed in the 10^{-9} dilution vial from the second dilution-to-extinction series whereas the dominant organism in the original DPH-1 culture was a short, thick rod (Fletcher et al., 2008).
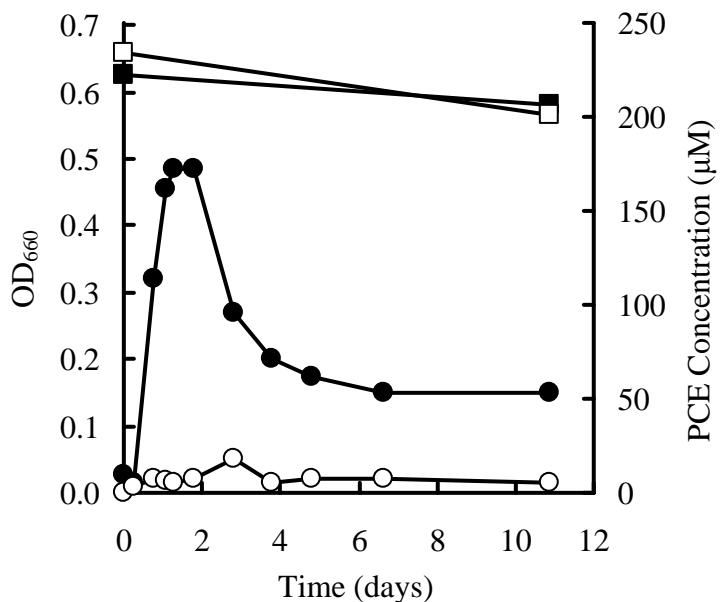
Figure 4-51. Growth in liquid cultures inoculated with isolated colonies from LB agar plates. Optical density (circles) and PCE concentration (squares) were monitored in live cultures (closed symbols) and cell-free controls (open symbols). Data were averaged from duplicate bottles and average variability within treatments was 26.8%.

Phylogenetic analysis confirmed the presence of two populations in culture DPH-1. Four 16S rRNA sequences obtained from the non-dechlorinating isolate (> 98.9% similarity) yielded a 1,369-bp consensus gene sequence that was 97.3% similar to the reported *Clostridium bifermentans* strain DPH-1 16S rRNA gene sequence (GenBank accession number Y18787.1). Alignment of the reported DPH-1 16S rRNA gene sequence with sequences from the non-dechlorinating isolate and the 10 most closely related sequences (accession numbers AY587782.1, AY587781.1, EF052864.1, AY167932.1, DQ978211.1, DQ218319.1, AY587793.1, AY167941.1, AF320283.1, and EF052865.1) demonstrated that the DPH-1 sequence included a 30-bp repeat from position 1068 to 1097 (*E. coli* numbering). The alignment revealed six additional incongruities between the reported DPH-1 16S rRNA gene sequence and the sequence from the non-dechlorinating *Clostridium* isolate (Table 4-10). These incongruities are thought to represent sequencing errors and/or sequence variability (Acinas et al., 2004; Shimizu, et al., 2001).

Table 4-10. Base pair differences between the reported 16S rRNA gene sequence of *Clostridium bifermentans* strain DPH-1 and the 16S rRNA gene sequences of the non-dechlorinating *Clostridium* isolate and 10 closely related sequences identified in GenBank by basic local alignment search tool (BLAST) analysis

E. coli Position	Reported DPH-1 Sequence	Sequence of Non-Dechlorinating Isolate	10 Most Similar Sequences
153	C	T	C (8) and T(2)
548	A	G	G
564-565	-[a]	CT	CT
654	C	T	T
726	A	G	G
921	C	T	T

[a] – indicates a deletion

The 16S rRNA gene sequence of the dechlorinating isolate was 99.6% similar (1,387 bp analyzed) to the 16S rRNA gene sequence of *Desulfitobacterium hafniense* strain Y51 (accession number AP008230.1), a known PCE-to-*cis*-DCE dechlorinating bacterium (Suyama et al., 2001). PCR with *Desulfitobacterium* spp. 16S rRNA gene-targeted primers (Lanthier et al., 2001) yielded an amplicon diagnostic for *Desulfitobacterium* with template DNA from the dechlorinating DPH-1 culture. Therefore, we propose that the organism responsible for PCE dechlorination in culture DPH-1 is a *Desulfitobacterium hafniense* strain, which was designated strain JH1.

Co-enrichment of PCE-dechlorinators with *Clostridium* spp. is not unprecedented. For example, Sung et al. (2003) reported a co-culture consisting of the PCE dechlorinator *Desulfuromonas michiganensis* strain BB1 and *Clostridium sphenoides*. Unexplored, and possibly symbiotic, nutritional interactions between *Clostridium* spp. and dechlorinators may exist. Understanding the interactions between dechlorinators and non-dechlorinating populations is relevant for successful bioremediation, emphasizing the need for detailed studies of the ecology of bacteria capable of respiratory reductive dechlorination (i.e., [de]chlororespiration).

4.3.1.2 Physiological Characterization of Strain JH1

Cultures of strain JH1 reduced (products given in brackets) PCE [*cis*-DCE] (Figure 4-52), TCE [*cis*-DCE], nitrate [ammonium], sulfite [sulfide], soluble Fe(III) [Fe(II)], and poorly crystalline Fe(III) oxide [Fe(II)]. Cultures amended with 1,1,2,2-tetrachloroethane formed 1,1,2-trichloroethane (22% mol/mol), *cis*-DCE (57%), and *trans*-DCE (21%). Under the conditions tested, strain JH1 did not reduce 1,1,2-trichloroethane, 1,1-dichloroethane (1,1-DCA), 1,2-DCA, *cis*-DCE, *trans*-DCE, vinyl chloride (VC), carbon tetrachloride, chloroform, dichloromethane, 1,2,3-trichloropropane, 1,2-dichloropropane, 2-chlorotoluene, hexachlorobenzene, 3-chloro-4-hydroxybenzoate, or sulfate. Strain JH1 used ethanol, formate, H$_2$, and pyruvate as electron donors, but acetate did not support reductive dechlorination under the conditions tested. Some *Desulfitobacterium* spp. have been reported to form spores (Villemur et al., 2006), but repeated efforts to recover activity from stationary phase strain JH1 cultures exposed to temperatures ranging from 60-80°C for 10 minutes were not successful.

Desulfitobacterium hafniense strain JH1 shares many physiological properties with strain Y51, but, in contrast to strain Y51, strain JH1 dechlorinated 1,1,2,2-tetrachloroethane to a mixture of 1,1,2-trichloroethane, *cis*-DCE, and *trans*-DCE rather than only to *cis*-DCE and used ethanol as an electron donor. Consistent with *Desulfitobacterium* physiology, strain JH1 failed to reduce sulfate whereas strain Y51 reportedly reduces sulfate (Suyama et al., 2001).
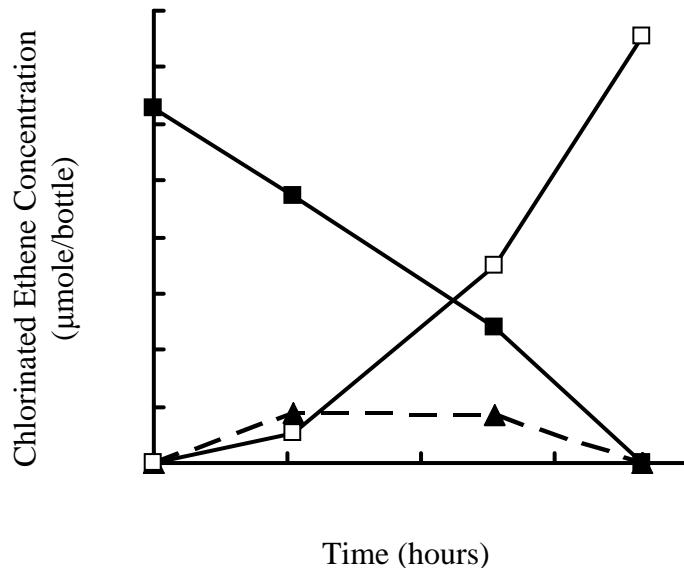
Figure. 4-52. Dechlorination of PCE (filled squares, solid line) to *cis*-DCE (open squares, solid line) with the intermediate formation of TCE (triangles, dashed line) by strain JH1. Data were averaged from duplicate cultures and average variability within cultures was 35.0%.

4.3.1.3 Identification of the PCE Reductive Dehalogenase Gene in Strain JH1

PCE reductive dehlaogenase (rdase) genes, *pceC* and *pceA*, have been identified in culture DPH-1 (Okeke et al., 2001) and *Desulfitobacterium hafniense* strain Y51 (Suyama et al., 2002), respectively. PCR was used to determine if either the *pceC* or *pceA* gene was present in strain JH1. Genomic DNA from strain JH1, the *Clostridium* isolate, and the mixed DPH-1 culture served as templates in separate PCR reactions, but none of the assays yielded an amplicon with primers designed to amplify the *pceC* gene. However, in reactions with primers designed to amplify the *pceA* gene, amplicons of the expected size (approximately 1,000 bp) were obtained with genomic DNA from strain JH1 and culture DPH-1. There was no visible amplification product produced with genomic DNA from the *Clostridium* isolate (Figure 4-53). The 935-bp sequence of the amplicon produced in the reaction with strain JH1 exactly matched the reported Y51 *pceA* gene sequence (accession number AP008230.1), suggesting that PCE dechlorination in culture JH1 is catalyzed by the *pceA* gene.
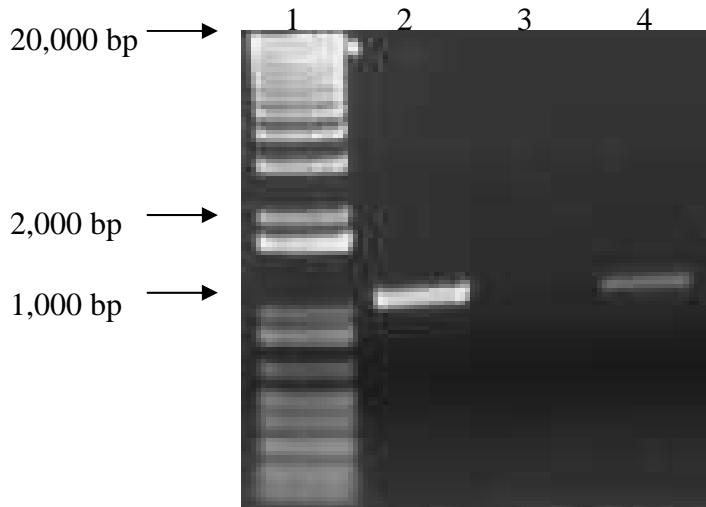
Figure 4-53. PCR amplification of the *pceA* gene. Lane 1, 1-kB plus size marker (Invitrogen); lane 2, strain JH1; lane 3, *Clostridium* isolate; lane 4, mixed DPH-1 culture.

4.3.2 The Effect of Elevated Temperatures on Reductive Dechlorination and Quantification of *Dehalococcoides* Biomarkers

While numerous organisms are capable of PCE and TCE dechlorination to *cis*-DCE (Gerritse et al., 1996; Holliger et al., 1998; Scholz-Muramatsu et al., 1995; Sung et al., 2006a; Sung et al., 2003), populations within the *Dehalococcoides* (*Dhc.*) genus are the only organisms known to reductively dechlorinate *cis*-DCE and VC (He et al., 2003a; He et al., 2005). In order to determine if *Dhc.* populations are present at contaminated sites, typically, DNA is extracted and populations are quantified via quantitative PCR (qPCR) with primers specific to the *Dhc.* 16S rRNA gene. However, a number of *Dhc.* strains have been isolated and characterized, revealing that even strains with highly similar or even identical 16S rRNA genes have unique substrate ranges (Ritalahti et al., 2006). Therefore, to assess the potential for specific dechlorination reactions *in situ*, qPCR primers have also been developed which target specific *Dhc.* rdase genes (Krajmalnik-Brown et al., 2004; Magnusen et al., 2000; Müller et al., 2004; Ritalahti et al., 2006). The *Dhc.* 16S rRNA gene as well as the reductive dehalogenase genes are termed *Dhc.* biomarkers (Amos et al., 2008). The goal of this study was to determine how elevated temperatures effect reductive dechlorination activity and quantification of *Dhc.* biomarkers. In addition, we aimed to determine if dechlorinating populations can recover activity following incubation at elevated temperatures.

4.3.2.1 Inhibition of Reductive Dechlorination at Elevated Temperatures

Bio-Dechlor INOCULUM (BDI) is a commercially available PCE-to-ethene dechlorinating culture that has been successfully applied for bioremediation of field sites (Ritalahti et al., 2005). BDI contains three unique *Dhc.* strains, strain BAV1, strain GT, and strain FL2 (Ritalahti et al., 2006). Both strain BAV1 and strain GT are capable of metabolic reductive dechlorination of VC to ethene (He et al., 2003a; Sung et al., 2006b), but strain FL2 is not (He et al., 2005). None of the *Dhc.* strains in BDI are capable of metabolic reductive

dechlorination of PCE and therefore the BDI culture contains a PCE-to-cis-DCE dechlorinating *Dehalobacter* population.

In BDI cultures amended with PCE and incubated at 30 ℃, complete dechlorination to ethene occurred within 30 days (Table 4-11, Figure 4-54A). In cultures incubated at 35℃, PCE was dechlorinated to VC and ethene, but even after 42 days of incubation VC persisted and accounted for $27.3 \pm 13.3\%$ (mol/mol) of dechlorination products (Figure 4-54B), indicating that VC dechlorination could not be sustained. In BDI cultures amended with PCE and incubated at 40℃, $17.6 \pm 3.5\%$ (mol/mol) of PCE was dechlorinated to VC within 2 days of PCE addition, but following this initial activity, dechlorination ceased and no ethene production occurred during incubation periods greater than 3 months. The presence of hydrogen in cultures incubated at 35 and 40 ℃ confirmed that the cessation in dechlorination activity was due to temperature effects on dechlorinating populations rather than electron donor limitations. The accumulation of VC during incubation at 35 ℃ demonstrates that *Dhc.* strain GT and strain BAV1 are inhibited during incubation at 35 ℃, while the PCE-to-*cis*-DCE and TCE-to-VC dechlorinating organisms, a *Dehalobacter* population and *Dhc.* strain FL2, respectively, are not.

In order to determine if both the *Dehalobacter* population and *Dhc.* strain FL2 are inhibited during incubation at 40 ℃, BDI cultures were amended with TCE and incubated at 40 ℃. Cultures dechlorinated TCE to VC and ethene, but, even after 49 days of incubation, VC persisted as $53.9 \pm 13.9\%$ of dechlorination products (Table 4-11), confirming that incubation at 40 ℃ is inhibitory to *Dhc.* strain BAV1 and strain GT. Additionally, these results demonstrate that *Dhc.* strain FL2 is not inhibited by incubation at 40 ℃, and, when compared to activity in cultures amended with PCE and incubated at 40 ℃, demonstrates that the *Dehalobacter* population is the dechlorinating organism inhibited by incubation at 40℃. In BDI cultures incubated at 45 ℃ and amended with TCE, TCE persisted after 49 days of incubation as $62.9 \pm 2.6\%$ of chlorinated ethenes and ethene, demonstrating that during incubation at 45 ℃, *Dhc.* strain FL2 is also inhibited (Table 4-11).
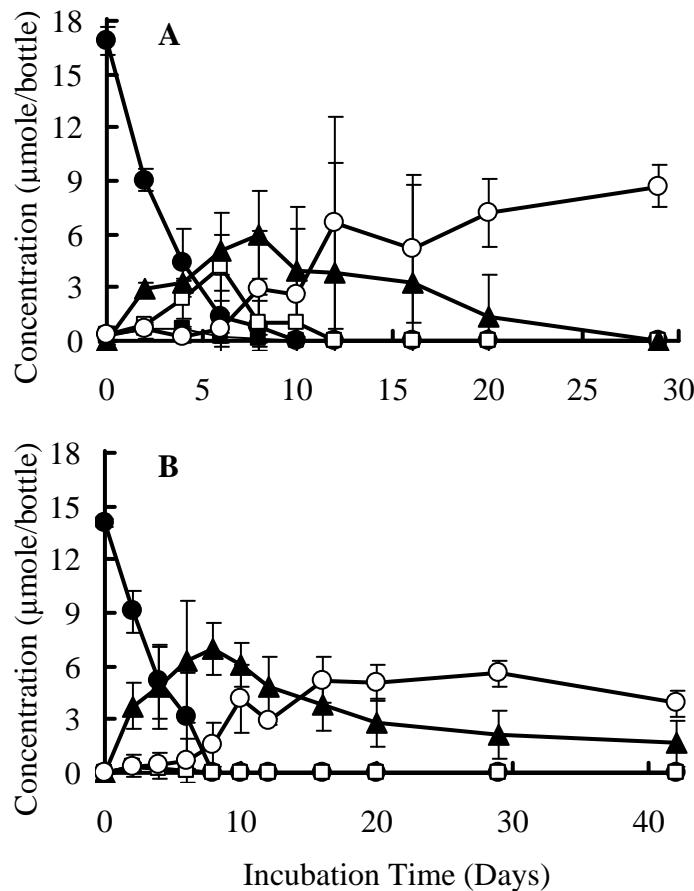
Figure 4-54. PCE (solid circles) dechlorination to TCE (solid squares), *cis*-DCE (open squares), VC (solid triangles), and ethene (open circles) in BDI cultures incubated at 30 ºC (A) and 35ºC (B). All data points represent average values from triplicate cultures and error bars depict one standard deviation.

Culture OW is a methanogenic PCE-to-ethene dechlorinating consortia that contains *Dehalobacter*, *Geobacter*, and *Sulfurospirillum* populations similar to organisms known to reduce PCE-to-*cis*-DCE (Holliger et al., 1998; Scholz-Muramatsu et al., 1995; Sung et al., 2006a). Culture OW also contains *Dhc*. populations and *Dhc*. biomarker genes, *tceA* and *vcrA* (Daprato et al., 2007). Results of experiments with the OW culture confirmed that *Dhc*. strains capable of VC dechlorination to ethene are more sensitive to elevated temperatures than those capable only of dechlorination to VC. Specifically, while OW cultures incubated at 30ºC completely dechlorinated PCE to ethene within 20 days from PCE amendment, OW cultures incubated at 35 and 40 ºC transformed PCE to VC, but produced no ethene even after 30 days of incubation (Table 4-11). In OW cultures incubated at 45 ºC, less than 5% of PCE (mol/mol) was converted to TCE and VC and no ethene was produced over the 30 day incubation period (Table 4-11). These results suggest that *Dhc*. populations may be universally inhibited at temperatures of 45 ºC and greater, but that strains responsible for dechlorination of VC to ethene are particularly sensitive to elevated temperatures.

Table 4-11. Dechlorination activity during incubation at elevated temperatures.

Culture	Provided Electron Acceptor	End Product[a]			
		30 ºC	35 ºC	40 ºC	45 ºC
BDI	PCE	Ethene	VC	PCE	Not tested
BDI	TCE	Not tested	Not tested	VC	TCE
OW	PCE	Ethene	VC	VC	PCE

[a] In cases where multiple end products were produced, the most chlorinated end product is listed.

4.3.2.2 Decreases in *Dehalococcoides* Cells During Incubation at Elevated Temperatures

Dhc. biomarker genes were quantified in DNA extracted from the BDI cultures amended with PCE and incubated at 30, 35, and 40 ºC (Figure 4-54, Table 4-11). DNA was extracted immediately after amendment with PCE and after 6 and 42 days of incubation. After 42 days of incubation, the concentration of *Dhc*. 16S rRNA gene copies was greatest in cultures incubated at 30 ºC, $2.51 \times 10^8 \pm 8.22 \times 10^7$ per ml culture, but *Dhc*. 16S rRNA gene copies were detected in all cultures at average concentrations of no less than $9.09 \times 10^7 \pm 6.63 \times 10^6$ copies per ml culture. Furthermore, even after this extended incubation time, there was no significant difference between the concentration of *Dhc*. 16S rRNA gene copies between cultures incubated at 30 ºC and in any other treatment (Figure 4-55A). *Dhc*. strain FL2 cells, quantified by *tceA* gene copies, were also detected in all cultures, but after 42 days of incubation, the concentration of FL2 cells in cultures incubated at 40 ºC, $4.02 \times 10^6 \pm 1.37 \times 10^6$ per ml culture, was significantly less than the concentration in cultures incubated at 30 ºC, $3.16 \times 10^7 \pm 2.53 \times 10^6$ per ml culture (Figure 4-55B).

After 42 days of incubation, *Dhc*. strain BAV1 cells, quantified by *bvcA* gene copies, were detected in cultures incubated at 30 ºC at $6.59 \times 10^4 \pm 6.38 \times 10^3$ per ml culture, but in cultures incubated at 35 and 40 ºC and under starvation conditions, BAV1 cells were below the detection limit of 9.54×10^3 copies per ml culture (Figure 4-55C). After only 6 days of incubation, the concentration of *Dhc*. strain GT cells, quantified by *vcrA* gene copies, was significantly greater in cultures incubated at 30 ºC, $1.61 \times 10^8 \pm 3.01 \times 10^6$ copies per ml culture, than in cultures incubated at 40 ºC, $9.08 \times 10^7 \pm 8.87 \times 10^6$ copies per ml culture. After 42 days of incubation, the concentration of GT cells in cultures incubated at 30 ºC, $2.11 \times 10^8 \pm 3.38 \times 10^7$ copies per ml culture, remained significantly higher than that in cultures incubated at 40 ºC, $7.29 \times 10^7 \pm 6.28 \times 10^6$ copies per ml culture. However, the concentration of GT cells in cultures incubated at 30ºC was also statistically greater than the concentration in starved control cultures, $7.26 \times 10^7 \pm 6.33 \times 10^6$ copies per ml culture (Figure 4-55D). Furthermore, there was no statistical difference between the concentration of GT cells in cultures incubated at 35 ºC, $8.92 \times 10^7 \pm 2.86 \times 10^7$ copies per ml culture, as compared to cultures incubated at 40 ºC and under starvation conditions.

These results corroborate the finding that *Dhc*. populations demonstrate strain-specific responses to exposure to elevated temperatures. Reductive dechlorination of VC to ethene was inhibited at incubation temperatures of 35 and 40 ºC (Table 4-11) and *Dhc*. strains capable of metabolic dechlorination of VC, strain BAV1 and strain GT, were either not detected or detected at lower concentrations in cultures incubated at 35 and 40 ºC than in cultures incubated 30 ºC. However, in both cases, after 42 days of incubation, the concentration of BAV1 and GT cells

were equivalent in cultures incubated at 35 and 40 ºC and in starved control cultures. This suggests that while incubation at elevated temperatures negatively impacts BAV1 and GT cells, this effect may be limited to temporary inhibition of activity. Therefore, BAV1 and GT cells may recover if temperatures cool.

Reductive dechlorination of TCE to VC was not inhibited in cultures incubated at 35 ºC and the *Dhc*. strain capable of metabolic dechlorination to VC in the BDI culture, strain FL2, was detected at similar concentrations in cultures incubated at 30 and 35 ºC. Due to the lack of PCE dechlorination to TCE in cultures incubated at 40 ºC (Table 4-11), FL2 cells lacked a metabolic electron acceptor in these cultures and therefore were incubated under starvation conditions. Because FL2 cells are not inhibited at incubation temperatures of 40ºC, (Table 4-11) it was expected that FL2 concentrations in these cultures would be similar to concentrations in starved control cultures. However, while FL2 concentrations were significantly lower in cultures incubated at 40 ºC than in cultures incubated at 30 ºC, there was no statistical difference between FL2 concentrations in cultures incubated at 30 ºC and those incubated under starvation conditions. This suggests that incubation at elevated, although not inhibitory, temperatures coupled to starvation conditions may cause greater effects than incubation under starvation conditions alone.

While these results demonstrate quantifiable strain-specific responses to incubation at elevated temperatures by *Dhc*. populations, the measured differences in DNA concentrations, even when statistically significant, are slight. Complete dechlorination of PCE to ethene only occurred in those cultures incubated at 30 ºC (Table 4-11), but *Dhc*. strains FL2 and GT were detected in all cultures and there was no significant difference in concentrations of total *Dhc*. 16S rRNA genes between cultures. Therefore, it is clear that other molecular techniques are necessary for the detection of *Dhc*. activity in situ.

*
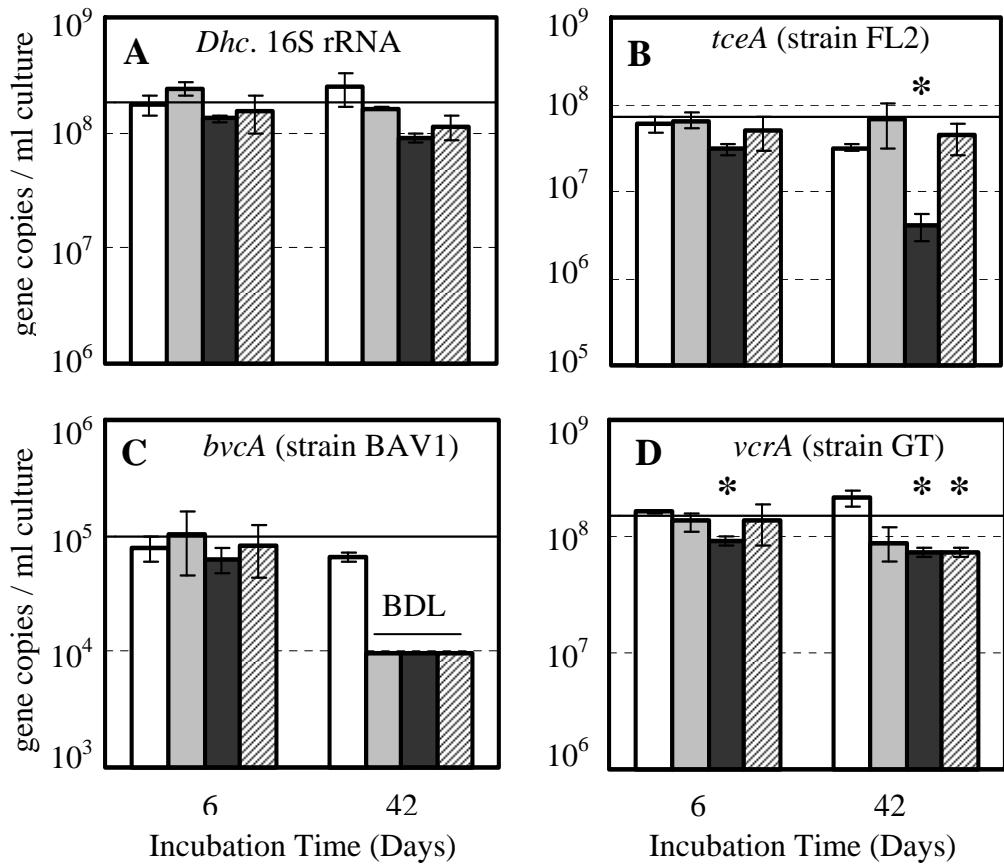
Figure 4-55. Average *Dhc.*16S rRNA (A), *tceA* (B), *bvcA* (C), and *vcrA* (D) gene copies per ml culture in cultures amended with PCE and incubated at 30 ℃ (open bars), 35℃ (gray bars), and 40 ℃ (filled bars) and in cultures incubated at 30 ℃, but not amended with PCE (striped bars). Dashed vertical lines are concentration gridlines and undashed vertical lines are average concentrations prior to incubation at elevated temperatures. Asterisks indicate concentrations that are significantly lower (p<0.01) than those in microcosms amended with PCE and incubated at 30 ℃.

4.3.2.3 Reductive Dehalogenase Transcription Does Not Correlate with Dechlorination Activity

Dhc. biomarker gene transcripts were quantified in RNA extracted from the BDI cultures amended with PCE and incubated at 30, 35, and 40 ℃ (Figure 4-54, Table 4-11). RNA was extracted immediately after amendment with PCE and after 6 and 42 days of incubation. Transcripts of the *bvcA* gene were not detected in any culture at any time. *Dhc*. 16S rRNA gene transcripts were detected in all cultures at all times, but after 6 days of incubation, the number of *Dhc*. 16S rRNA gene transcripts per *Dhc*. cell in cultures incubated at 35 and 40 ℃ were 2.92 ± 0.35 and 3.34 ± 0.30 orders-of-magnitude higher, respectively, than in cultures incubated at 30 ℃ (Table 4-12). Conversely, the number of *Dhc*. 16S rRNA gene transcripts per *Dhc*. cell in starved control cultures was approximately equivalent to the number of transcripts per cell in

cultures incubated at 30 ℃. After 42 days of incubation, *Dhc*. 16S rRNA gene transcripts per *Dhc*. cell were approximately equivalent in all treatments (Figure 4-56A).

After 6 days of incubation, the average number of *tceA* gene transcripts per FL2 cell was 2.82 ± 0.44 orders-of-magnitude higher in cultures incubated at 35 ℃ than in positive control cultures incubated at 30 ℃ (Table 4-12) even though the *tceA* gene catalyzed TCE dechlorination to VC in both cultures incubated at 30 and 35 ℃ (Table 4-11). In cultures incubated at 40 ℃, *tceA* gene transcripts per FL2 cell were within an order of magnitude of concentrations in cultures incubated at 30 ℃ (Table 4-12) even though cultures incubated at 40 ℃ were actually incubated under starvation conditions as no TCE was produced from PCE dechlorination in these cultures (Table 4-11). In starved control cultures, *tceA* gene transcripts were not detected after 6 days of incubation. Assuming that the concentration of gene transcripts was the *tceA* detection limit, 3.16×10^2 *tceA* gene transcripts per ml, there were a minimum of 1.30 ± 0.29 orders-of-magnitude fewer *tceA* gene transcripts per FL2 cell in starved control cultures than in cultures incubated at 30 ℃. After 42 days of incubation, the average number of *tceA* gene transcripts per FL2 cell was within one order-of-magnitude in all treatments (Figure 4-56B).

After 6 days of incubation, the number of *vcrA* gene transcripts per GT cell in cultures incubated at 35 and 40 ℃ were 2.65 ± 0.47 and 2.14 ± 0.52 orders-of-magnitude higher, respectively, than in cultures incubated at 30 ℃ (Table 4-12) even though these cultures did not demonstrate reductive dechlorination of VC (Table 4-11). The number of *vcrA* gene transcripts per GT cell in starved control cultures was within an order-of-magnitude of the number in cultures incubated at 30 ℃. After 42 days of incubation, the average number of *vcrA* gene transcripts per GT cell was within an order-of-magnitude in all treatments (Figure 4-56C).

In general, after 6 days of incubation, transcription of the *Dhc*. 16S rRNA gene, *tceA* gene, and *vcrA* gene was greater in cultures incubated at 35 and 40 ℃ as compared to in cultures incubated at 30 ℃. The upregulation of the *vcrA* gene by more than 2 orders-of-magnitude in cultures incubated at 35 and 40℃ as compared to in cultures incubated at 30 ℃ is particularly remarkable as reductive dechlorination of VC to ethene is inhibited at temperature of 35 ℃ and greater.   After 6 days of incubation, transcription in starved control cultures was either approximately equivalent to or less than in positive control cultures incubated at 30℃. After 42 days of incubation, transcription in all culture was within an order-of-magnitude of transcription in positive control cultures incubated at 30 ℃.

Results from RNA extraction and quantification of gene transcripts per cell demonstrate differences between cultures greater than an order-of-magnitude within 6 days of incubation, but only minor differences after 42 days of incubation. Conversely, results from DNA extraction and quantification of gene concentrations demonstrated few order-of-magnitude differences and the majority of significant differences in gene concentrations were resolved after 42 days of incubation rather than after 6. These results are unsurprising since RNA gene transcripts likely turnover faster than DNA and therefore vary more dramatically over shorter time periods. In addition, the number of gene transcripts per cell may have been similar after 42 days of incubation because active dechlorination had ceased in all culture sets either due to complete dechlorination to ethene or inhibition of dechlorination activity (Figure 4-54). Unfortunately, unlike the trends demonstrated by quantification of gene concentration in extracted DNA, gene transcript numbers quantified from extracted RNA did not vary predictably with dechlorination activity. In fact, over short time periods, orders-of-magnitude more gene transcripts were

detected in cultures incubated at elevated temperatures than in positive control cultures incubated at 30 ºC, even when dechlorination activity was inhibited in cultures incubated at elevated temperatures. These results corroborate those of Amos et al. (2008), demonstrating that even when reductive dechlorination is inhibited, *Dhc*. gene transcripts may be detected and therefore, the use of RNA biomarker analysis for estimation of *in situ* activity has limitations.

Table 4-12. Summary of transcripts per cell relative to positive control cultures amended with PCE and incubated at 30 ºC.

Culture	**Log Transcripts per Cell Relative to Positive Control**[a]								
	***Dhc*. 16S rRNA**			***tceA***			***vcrA***		
6 Days of Incubation									
35	2.92	±	0.35	2.82	±	0.44	2.65	±	0.47
40	3.34	±	0.30	0.54	±	0.29	2.14	±	0.52
Starved Control	-0.07	±	0.37	<-1.30[b]	±	0.29	-0.59	±	0.56
42 Days of Incubation									
35	0.02	±	0.15	-0.42	±	0.16	-0.10	±	0.40
40	0.06	±	0.14	0.93	±	0.71	-0.12	±	0.49
Starved Control	0.10	±	0.19	-0.34	±	0.40	-0.44	±	0.33

[a] Log gene transcripts per cell (e.g. *vcrA* gene transcripts divided by *vcrA* gene copies) relative to the number of transcripts per cell in positive control cultures incubated at 30 ºC and amended with PCE.

[b] Because *tceA* gene transcripts were below the detection limit, it was assumed that the number of *tceA* gene transcripts was the detection limit of 3.16×10^2 transcripts per ml culture.
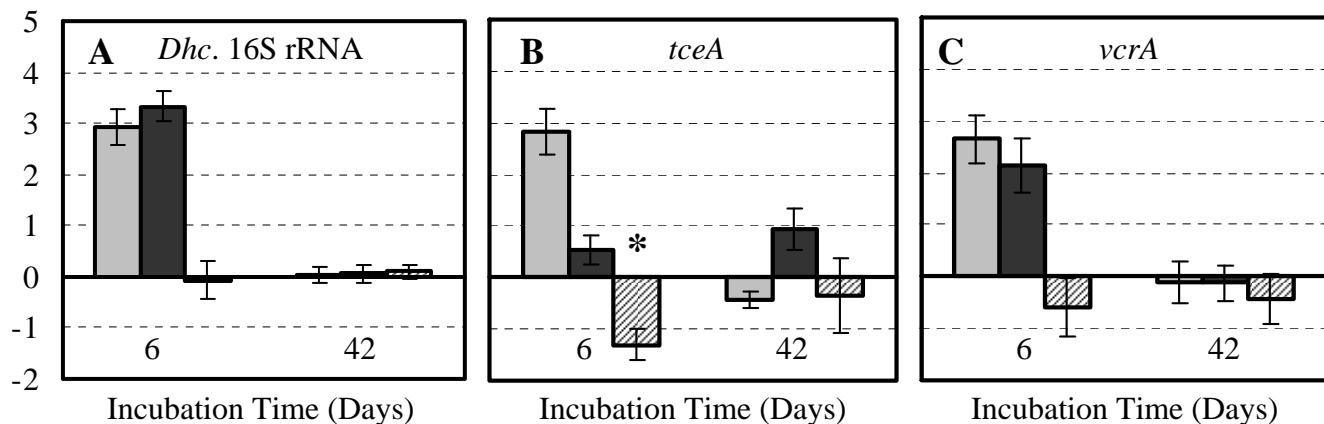
Figure 4-56. Log average *Dhc.* 16S rRNA (A), *tceA* (B), and *vcrA* (C), gene transcripts per cell in cultures amended with PCE and incubated at 35 ºC (gray bars) and 40 ºC (filled bars) and in cultures incubated at 30 ºC, but not amended with PCE (striped bars) normalized to transcripts per cell in cultures amended with PCE and incubated at 30 ºC. The asterisk indicates that *tceA*

transcripts were below the detection limit in this sample and therefore were assumed to be the detection limit, 3.16×10^2 transcripts per ml culture.
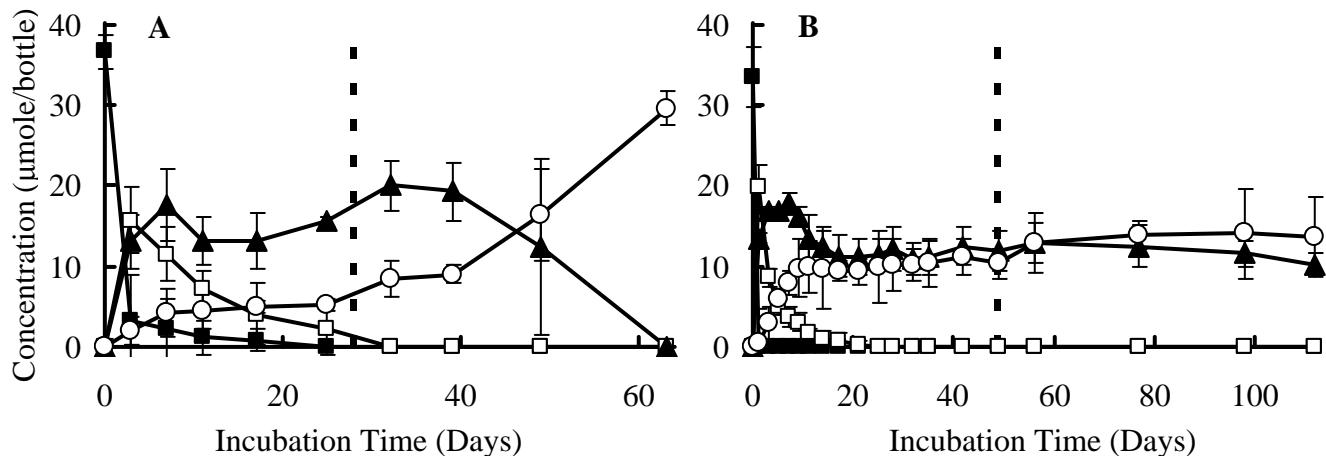
Figure 4-57. TCE (solid squares) dechlorination to *cis*-DCE (open squares), VC (solid triangles), and ethene (open circles) in BDI cultures incubated at 40 ºC for 28 (A) and 49 days (B). The vertical dashed line indicates the decrease in incubation temperature from 40 to 24 ºC. All data points represent average values from triplicate cultures and error bars depict one standard deviation.

4.3.2.4 Recovery of Dechlorination Activity Following Cooling from Inhibitory Temperatures

In order to determine if *Dhc.* strain BAV1 and strain GT are capable of recovering VC dechlorination activity after incubation at elevated temperatures, triplicate BDI cultures were amended with TCE and incubated at 40ºC prior to cooling to 24ºC. During incubation at 40ºC, TCE was dechlorinated to VC and ethene, but VC persisted in all cultures, accounting for 58.0 ± 13.5% (mol/mol) of dechlorination end products prior to cooling. After cultures were cooled to 24ºC, VC was completely dechlorinated to ethene in cultures incubated at 40ºC for 7, 14, and 28 days. However, as incubation time at 40ºC increased, the time for complete dechlorination to ethene also increased. Specifically, in cultures incubated at 40ºC for 7 days, VC was completely dechlorinated to ethene 25 days after cooling whereas in cultures incubated at 40ºC for 14 and 28 days, VC was completely dechlorinated to ethene within 28 and 35 days, respectively (Figure 4-57A). In cultures incubated at 40ºC for 49 days, VC was not dechlorinated to ethene even after 63 days of incubation at 24ºC (Figure 4-57B). Previous experiments have verified that reductive dechlorination to ethene can recover in BDI cultures following starvation conditions lasting longer than 49 days, demonstrating that in this case, recovery did not fail due to the effects of starvation conditions alone. These results indicate that even after extended incubation times, VC reductive dechlorination activity can be recovered, but that recovery time increases with increasing exposure time to elevated temperatures. Furthermore, recovery may not occur after exposure times of greater than 28 days.

126

4.3.3 Electron Donor Availability Following Thermal Treatment

It has been reported that thermal treatment releases organic carbon from the subsurface matrix, increasing electron donor availability for dechlorinating organisms (Friis et al., 2005; Friis et al., 2006). However, the released organic carbon, suggested to be in the form of long-chain fatty acids, cannot directly serve as an electron donor for *Dhc*. spp. Friis et al. (2005; 2006) suggested that the organic carbon released during thermal treatment may be fermented, causing a release of hydrogen, and that, therefore, biostimulation of soils following thermal treatment may not be required. In fact, in previous microcosm studies performed by Friis et al. (2006) complete dechlorination of TCE to ethene occurred in 2 out of 3 heated microcosms both with and without electron donor addition. Conversely, in the same study, while complete dechlorination to ethene occurred in all unheated and biostimulated microcosms, complete dechlorination occurred in only 1 of 3 unheated microcosms that were not biostimulated. While these results suggest that biostimulation may not be required to promote complete dechlorination to ethene following thermal treatment, in this previous study, initial hydrogen concentrations in the majority of microcosms were at least three orders-of- magnitude greater than concentrations typically found in groundwater (Chapelle et al., 1996). Another potential beneficial effect of thermal treatment may be that hydrogen-consuming competitors to dechlorinating population will not recover from elevated temperatures. Common competitors to dechlorinating populations are methanogens, most of which consume hydrogen. Active methanogens compete for electron donor with dechlorinating organisms and may contribute to aquifer clogging (Friis et al., 2006). In a previous study, less methanogenesis was observed in heated microcosms as compared to unheated microcosms even after cooling and bioaugmentation with a methanogenic culture, suggesting that competitors to dechlorinating populations are less successful following thermal treatment (Friis et al., 2006).

The goal of this study was to determine how thermal treatment affects the availability of electron donors for bioaugmented dechlorinating organisms. To determine the affect of incubation temperature on dechlorination activity and electron donor consumption, triplicate microcosm sets were constructed with soils from a PCE contaminated site, incubated at 24, 35, 50, 70, and 95 ℃, cooled, and bioaugmented. Reductive dechlorination and methanogenic activity were compared across microcosm sets to determine if competitors to dechlorinators significantly impacted electron donor availability and if the success of dechlorinators and/or their competitors correlated with previous incubation temperature.

4.3.3.1 Chlorinated Ethene Concentrations Prior to Bioaugmentation

In all microcosems, PCE concentrations decreased with incubation time. Following cooling, PCE concentrations were inversely proportional to previous incubation temperature ($r^2 = 0.84$) with 23.9 ± 1.7 µmole PCE in microcosms previously incubated at 24 ℃, 17.3 ± 1.0 at 35 ℃, 11.4 ± 0.9 at 50 ℃, 12.6 ± 0.6 at 70 ℃, and 6.2 ± 1.1 at 95 ℃. This decrease in PCE concentration could be accounted for by biotic reductive dechlorination, abiotic degradation, or loss of PCE from the microcosm. However, similar decreases were observed in the abiotic control microcosms and degradation products such as *cis*-DCE, VC, ethene, and acetylene were not detected. Trace concentrations (< 0.1 µmole/bottle) of TCE were measured, but

concentrations did not increase with incubation time and were similar in biotic and abiotic controls. Because trace amounts of TCE were initially present in the Great Lakes clay slurry, the decrease in PCE concentrations is primarily due to diffusion through the rubber stopper rather than biotic or abiotic degradation. This conclusion is consistent with a previous report that demonstrated that PCE loss in vials sealed with polymer septa and incubated at 50 ºC was mainly due to diffusion (Costanza and Pennell, 2007c). Because no native organisms capable of reductive dechlorination were active in any of the microcosms, bioaugmentation was required for complete dechlorination of PCE to ethene.

4.3.3.2. Reductive Dechlorination Following Bioaugmentation

To determine if thermal treatment promotes microbial reductive dechlorination, after cooling to 24 ºC, microcosms were bioaugmented with the methanogenic PCE-to-ethene-dechlorinating OW culture. At least 95% of PCE was dechlorinated to *cis*-DCE or VC only three days after bioaugmentation. No significant increase in the mole percent of VC occurred in any microcosm set from 11 to 20 days following bioaugmentation, demonstrating that dechlorination activity had ceased (Figure 4-58). Because hydrogen concentrations were below the detection limit of 40 ppmv in all microcosms 11 days after bioaugmentation, the accumulation of VC and the stall in reductive dechlorination activity may have been due to electron donor limitation. While it was proposed that heating soils increases bioavailable organic carbon concentrations, thus providing substrate(s) for hydrogen-releasing fermentation reactions (Friis et al., 2005; 2005), the results with Great Lakes soil microcosms shows that even following heating there was insufficient hydrogen present to suport the VC to ethene metabolic pathway.
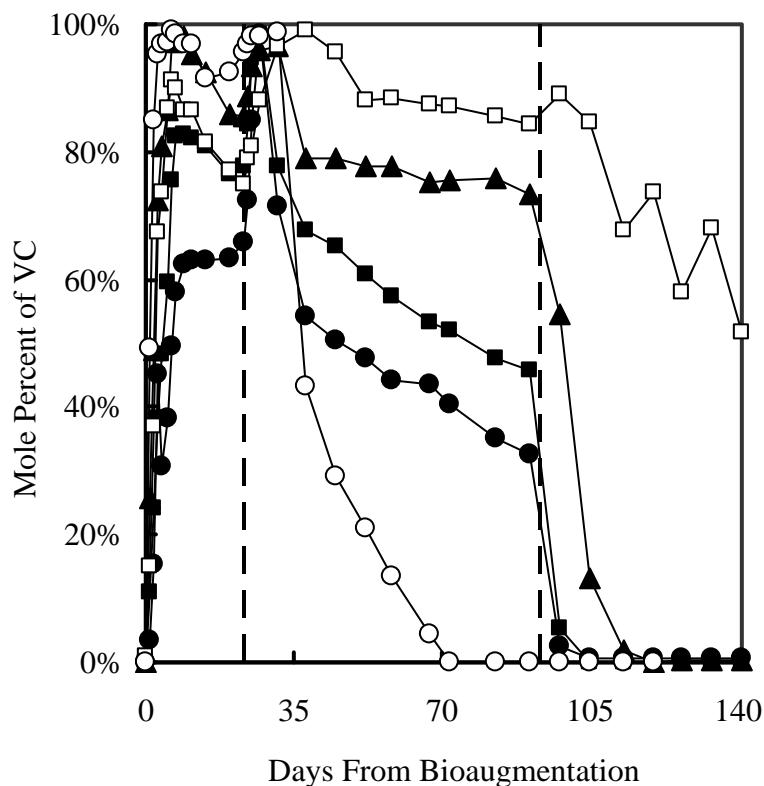
Figure 4-58. Mole percent of VC in microcosms previously incubated at 24 ℃ (filled circles), 35 ℃ (filled squares), 50 ℃ (triangles), 70℃ (open squares), and 95 ℃ (open circles). Vertical dashed lines indicate biostimulation events.

After 20 days of incubation, the mole percent of VC produced did not strongly correlate with previous incubation temperature ($r^2 = 0.66$) and the mole percent of VC in microcosms previously incubated at 95 ℃ was not significantly greater than that in the microcosms previously incubated at 24, 35, or 50 ℃. However, initial PCE concentrations varied inversely with previous incubation temperature and therefore, microcosms previously incubated at 95 ℃ contained less initial PCE and required fewer electron equivalents for dechlorination to VC than microcosms incubated at lower temperatures. In order to determine if electron donor availability varied with previous incubation temperature, dechlorination activity was normailzed based on consumption of electron equivalents (Figure 4-59). The number of electron equivalents consumed for dechlorination 20 days after bioaugmentation was significantly lower in those microcosms previously incubated at 95 ℃ as compared to every other microcosm set. Furthermore, the number of electron equivalents consumed for reductive dechlorination decreased linearly with increasing previous incubation temperature ($r^2 = 0.92$). These results suggest that either less electron donor was available in the microcosms previously incubated at 95 ℃ or that the available electron donor was consumed by competitors to dechlorinating organisms.
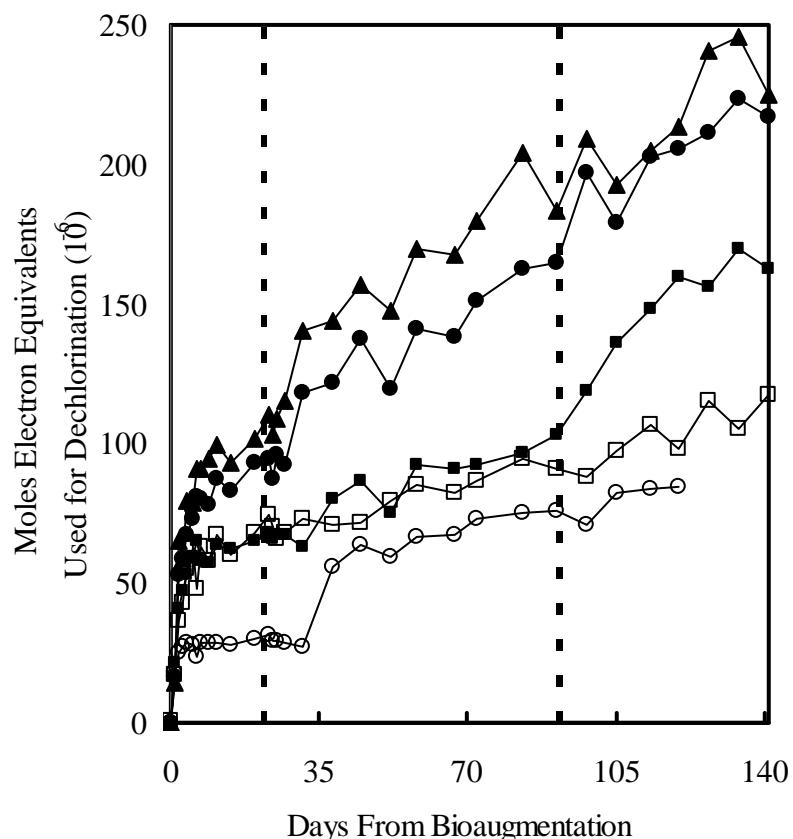
Figure 4-59. Electron equivalents consumed for reductive dechlorination in micrcosms previously incubated at 24 ℃ (triangles), 35 ℃ (filled circles), 50 ℃ (filled squares), 70 ℃ (open squares), and 95 ℃ (open circles). Vertical dashed lines indicate biostimulation events.

4.3.3.3 Reductive Dechlorination Following Biostimulation

Because dechlorination activity ceased prior to complete dechlorination to ethene, all microcosms were biostimulated with 5 mL of hydrogen gas. Within 8 days of biostimulation, at least 95% of chlorinated ethenes were dechlorinated to VC or ethene in all microcosms (day 31, Figure 4-58) the number of electron equivalents consumed for reductive dechlorination increased by at least 14% in all microcosm sets within 38 days of biosimulation (day 61, Figure 4-59). The increase in reductive dechlorination activity following biostimulation supports the conclusion that the previous stall in dechlorination activity was caused by electron donor limitation.

The mole percent of VC was significantly lower in microcosms previously incubated at 95 °C as compared to all other microcosm sets 68 days after biostimulation (day 91, Figure 4-58), indicating increased VC reduction and ethene formation in the microcosms previously incubated at 95 °C. However, a significantly higher mole percent of VC was converted to ethene in microcosms incubated at 24 °C than in microcosms previously incubated at 50 and 70°C. Thus, the mole percent of VC did not correlate strongly to previous incubation temperature ($R^2 = 0.06$), similar to results following biostimulation. Following bioaugmentation, the number of electron equivalents consumed for reductive dechlorination decreased with previous incubation temperature. Conversely, following biostimulation, the number of electron equivalents consumed did not correlate strongly with previous incubation temperature ($r^2 = 0.48$). This suggests that upon the introduction of excess electron donor, previous incubation temperature no longer effected electron donor consumption for reductive dechlorination.

The mole percent of VC did not signficantly decrease in any microcosm set from 60 to 68 days following biostimulation (days 83 to 91, Figure 4-58) and hydrogen was not detected in any microcosm 68 days after biostimulation. Therefore, in order to achieve complete detoxification to ethene, microcosms were amended with 5 mL of hydrogen gas twice per week (aggressive biostimulation) beginning 93 days after bioaugmentation. Within 30 days of this aggressive biostimulation, VC concentrations were below the detection limit of 0.2 mg/L in all microcosms with the exception of the microcosms previously incubated at 70 °C (day 123, Figure 4-58). Of the three microcosms previously incubated at 70 °C, VC concentrations in two of the microcosms did not significantly decrease during biostimulation. In the third microcosm, the mole percent of VC decreased from 78.0% to 7.1% over 50 days of aggressive biostimulation. Based on these results, while aggressive biostimulation effectively fueled VC reduction to ethene in most microcosms, reductive dechlorination was significantly inhibited in the microcosms previously incubated at 70 °C.
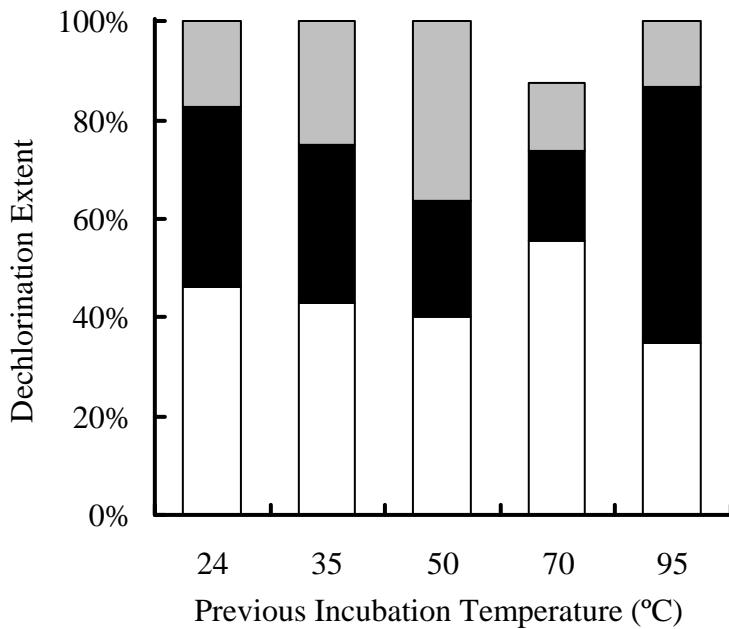
Figure 4-60. Dechlorination extent following each treatment as measured by electron consumption.

In order to quantify the effect of biostimulation on reductive dechlorination, the dechlorination extent was determined following each treatment. Dechlorination extent was defined as the number of electron equivalents used for reductive dechlorination normalized to those required for complete dechlorination to ethene. The dechlorination extent prior to biostimulation was significantly greater in those microcosms incubated at 24 and 35 ℃ as compared to microcosms previously incubated at 95 ℃ (Figure 4-60). Following biostimulation, the dechlorination extent increased significantly in all microcosm sets and was not significantly different between microcosms previously incubated at 24 ℃ and at any other temperature. The maximum dechlorination extent in microcosm sets following biostimulation was 87% and increased to 100% in all microcosm sets, (except those previously incubated at 70 ℃), after aggressive biostimulation. This demonstrates that aggressive biostimulation was required for complete reductive dechlorination to ethene.

These results confirm that: a) biostimulation significantly increased dechlorination extent regardless of previous microcosm incubation temperature, b) prior to biostimulation, dechlorination extent and electron donor consumption for reductive dechlorination were significantly greater in microcosms previously incubated at 24 and 35 ℃ than in microcosms previously incubated at 95 ℃) following biostimulation, previous incubation temperature did not effect dechlorination extent or electron donor consumption for reductive dechlorination. While this study has concluded that biostimulation was required for complete reductive dechlorination in Great Lakes soil microcosms regardless of previous incubation temperature, Friis et al. (2006) reported that biostimulation had no effect on dechlorination end-products in microcosms previously heated to 100 ℃. The inconsistency between these results may be due to either site-specific differences in the amount of electron donor released during thermal treatment or due to the exclusion of exogenous hydrogen in the present study.

131

4.3.3.4 Competition for Electron Donors

Methanogens compete with dechlorinating organisms for hydrogen, which is the only known electron donor for microbial reductive dechlorination of *cis*-DCE and VC. Prior to bioaugmentation, methane was not produced in any microcosm, suggesting that methanogenic archaea were not active in Great Lakes soil and groundwater. However, after bioaugmentation, average methane concentrations in all microcosm sets were above 50,000 ppmv (Figure 4-61). Because no methane was present prior to bioaugmentation, it is likely that methane was produced by the methanogens introduced with the bioaugmentation inoculum, culture OW. The ratio of the number of electron equivalents consumed for methanogenesis to electron equivalents consumed for reductive dechlorination prior to biostimulation was a minimum of 2,500, indicating that in all microcosm sets less than 0.04% of the available reducing equivalents were consumed for reductive dechlorination. Methane concentrations in microcosms previously incubated at 95 ℃ were significantly greater than concentrations in those microcosms previously incubated at 24 and 35 ℃, suggesting that heat treatment increased substrate availability for methanogens. Furthermore, prior to biostimulation, the ratio of the number of electron equivalents used for methanogenesis to those used for reductive dechlorination was significantly greater in those microcosms previously incubated at 95 ℃ as compared to all other microcosm sets. In fact, the ratio of the number of electron equivalents used for methanogenesis to electron equivalents used for reductive dechlorination increased linearly with previous incubation temperature (r^2 = 0.80). Because more methane was produced and the ratio of electron equivalents consumed for methanogenesis to reductive dechlorination was significantly greater in the microcosms previously incubated at 95 ℃ as compared to microcosms previously incubated at 24 ℃, it is evident that the increase in electron donor caused by thermal treatment in Great Lakes soils did not stimulate reductive dechlorination but rather methanogenesis.

Following biostimulation, significantly more methane was produced and the ratio of electron equivalents used for methanogensis to reductive dechlorination was significantly higher in microcosms previously incubated at 24 ℃ as compared to microcosms previously inubated at 95 ℃ (Figure 4-61). These results suggest that while methanogenesis was limitited by insufficient electron donors prior to biostimulation in microcosms previously incubated at 24 ℃, methane production recovered upon the introduction of additional electron donor. During aggressive biostimulation, average methane concentrations in all microcosm sets increased by at least 22%. Furthermore, the ratio of electron equivalents used for methanogenesis to electron equivalents consumed for reductive dechlorination was a minimum of 6,300, indicating that less than 0.02% of electron equivalents were consumed for reductive dechlorination. Although the ratio of electron equivalents consumed for methanogenesis to those consumed for reductive dechlorination was higher in microcosms previously incubated at 95 ℃ than in all other microcosm sets, this can likely be attributed to the lower initial PCE content, meaning that there were fewer electron equivalents required to achieve complete dechlorination to ethene in these microcosms. During aggressive biostimulation, there was no significant difference in the amount of methane produced between microcosms previously incubated at 24 ℃ and all other microcosm sets. These results corroborate that while methanogenesis was initially inhibited in microcosms previously incubated at 24 ℃, methane production recovered upon the introduction of additional electron donor.

Interestingly, during the period following biostimulation and prior to aggressive biostimulation, no methane was produced in the microcosms previously incubated at 50 ºC (Figure 4-61). Therefore, during this time, the ratio of electron equivalents used for methanogenesis to electron equivalents consumed for reductive dechlorination was significantly lower in microcosms previously incubated at 50 ºC than in all other microcosm sets. Furthermore, the total average ratio of electron equivalents used for methanogenesis to reductive dechlorination in microcosms previously incubated at 50 ºC was below 30,000, more similar to the values in the microcosms incubated at 24 and 35 ºC than those incubated at 70 and 95 ºC. This suggests that thermal treatment at intermediate temperatures (e.g., 50 ºC) rather than elevated temperatures (e.g., 95 ºC) may result in less competition for electron donors following biostimulation. Even so, regardless of previous incubation temperature, the vast majority of available electron donor was consumed for methanogenesis rather than reductive dechlorination both prior to and following biostimulation. Therefore, bioaugmentation with cultures that do not contain methanogens may be advantageous.
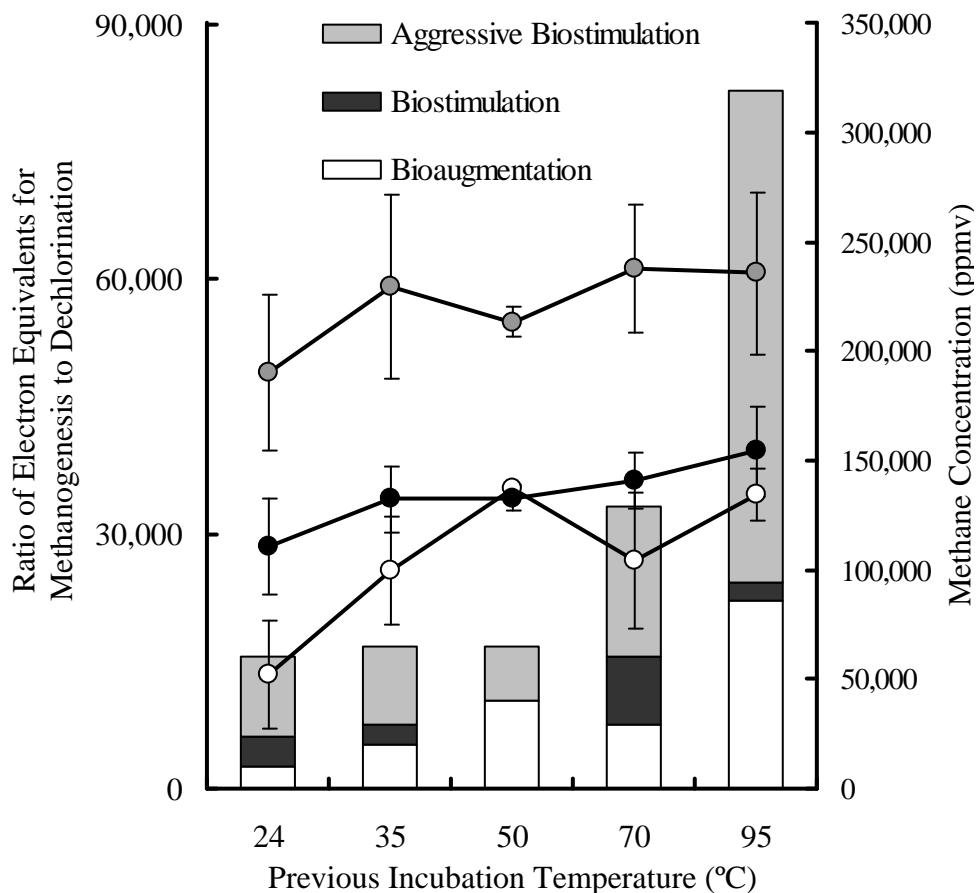
Figure 4-61. Ratio of electron equivalents used for methanogenesis to electron equivalents used for reductive dechlorination (bars) and methane concentrations (lines) following each treatment.

4.4. THERMAL TREATMENT PERFORMANCE EVALUATION

A 2-D ERH treatment cell described in Methods Section 3.6 was used for three experiments; one involving ERH treatment of the soil (i.e., ERH alone), a second experiment involving ERH combined with persulfate to promote in-situ oxidation of PCE, and a third experiment involving ERH combined with bioaugmention to transform the PCE emanating from the source zone into ethene.

4.4.1. ERH Treatment

An initial experiment was completed by treating Great Lakes soil collected from Borehole F3 between 8 and 10 feet bgs with ERH to determine if passing electrical current through the soil would result in PCE degradation. Once heated to 74 °C, the concentration of PCE in the groundwater after passing through the heated zone increased from below the 0.2 mg/L detection limit to 106 ± 16 mg/L, demonstrating the increase in PCE mass transferred to the aqueous phase caused by heating the contaminated soil (Figure 4-62). The concentration of PCE then began to decline, reaching ca. 30 mg/L after 4.1 L of groundwater or approximately 6.3 pore volumes had passed through the column. The temperature was increased to 88 °C at this point to determine if more PCE mass could be recovered, and there was a slight increase in PCE concentrations to ca. 70 mg/L. After this point, the PCE concentrations again declined reaching ca. 10 mg/L after 8.6 L of water had passed through the column. A temperature increase to 100 °C caused another temporary increase in PCE concentration, but also caused all influent water to be transformed into gas phase, which resulted in loss of hydraulic control and drying out of the electrodes. The Great Lakes soil temperature was returned to 88 °C so that water was the primary phase in the column in order to maintain electrical conductivity. The PCE concentrations continued to decline, reaching ca. 1 mg/L after 14 L of water had passed through the column. The temperature was again increased, this time to 95 °C to avoid drying out the heated zone, and this last increase had minimal effect on the concentration of PCE with a slight increase from ca. 1 mg/L to 2 mg/L at 16.6 L of water through the column.
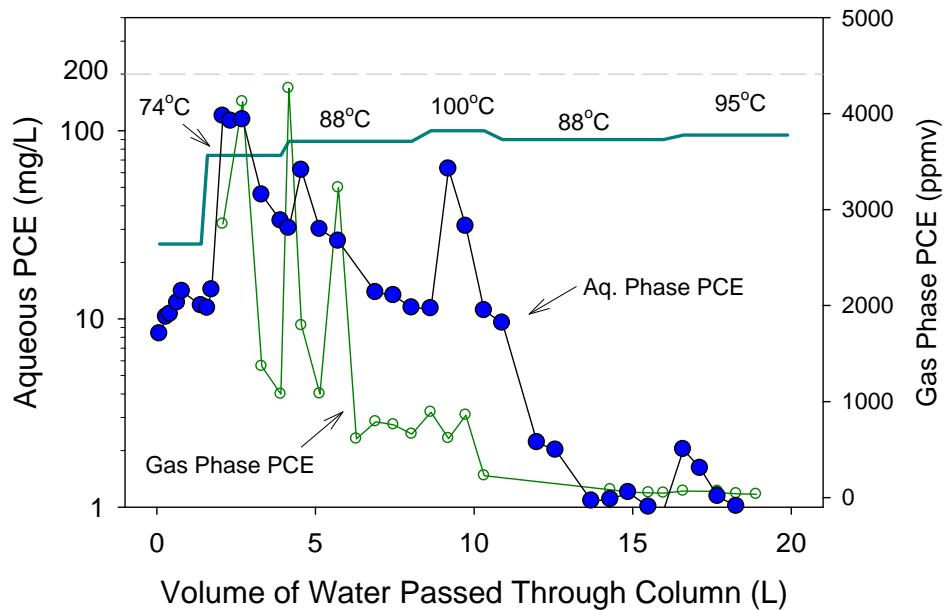
Figure 4-62. Concentration of PCE in aqueous and gas phase during ERH treatment.
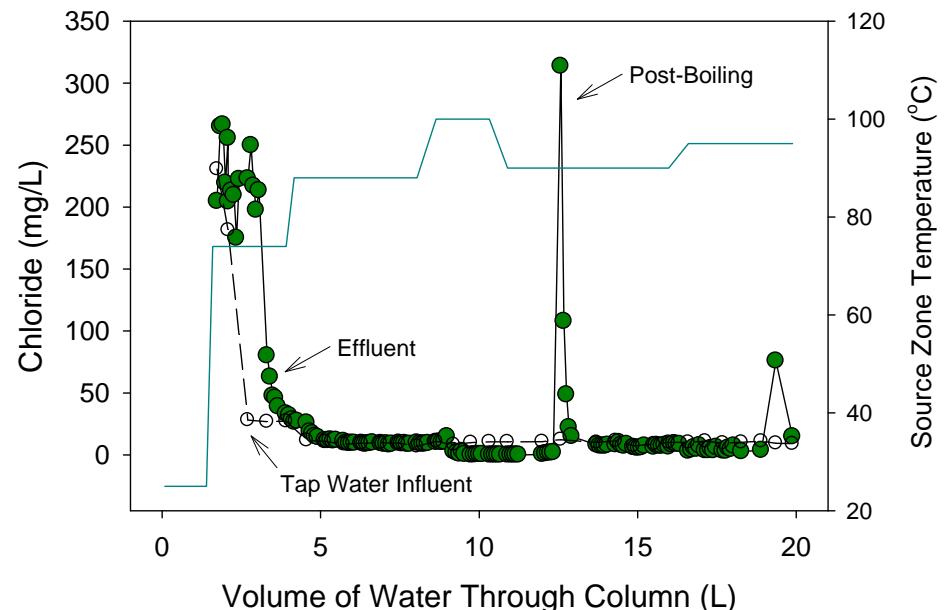
Figure 4-63. Concentration of chloride in aqueous phase during ERH treatment.

While PCE concentrations increased from 13 to 106 mg/L during heating, chloride levels decreased from 200 mg/L to 10 mg/L as resident groundwater was replaced with the tap water used to maintain soil moisture (Figure 4-63). If PCE were being dechlorinated during ERH treatment, then an increase in chloride was expected. There were two increases in chloride

observed, one occurred after decreasing the heated zone temperatures from 100 to 88 °C, which was done to restore water flow through the column. This short-term increase in chloride levels to 314 mg/L for less than one pore-volume in duration may have represented PCE degradation or chloride that precipitated in the heated zone when gas flow predominated at 100°C. Without water flowing through the column, chloride would remain at the point where water was transformed into vapor, similar to the salt buildup that has to be periodically removed from steam generators. The second short-duration increase in chloride was near the end of the experiment after 19 L of water had passed through the column and PCE concentrations were near 1 mg/L. The cause of this second increase is unknown.
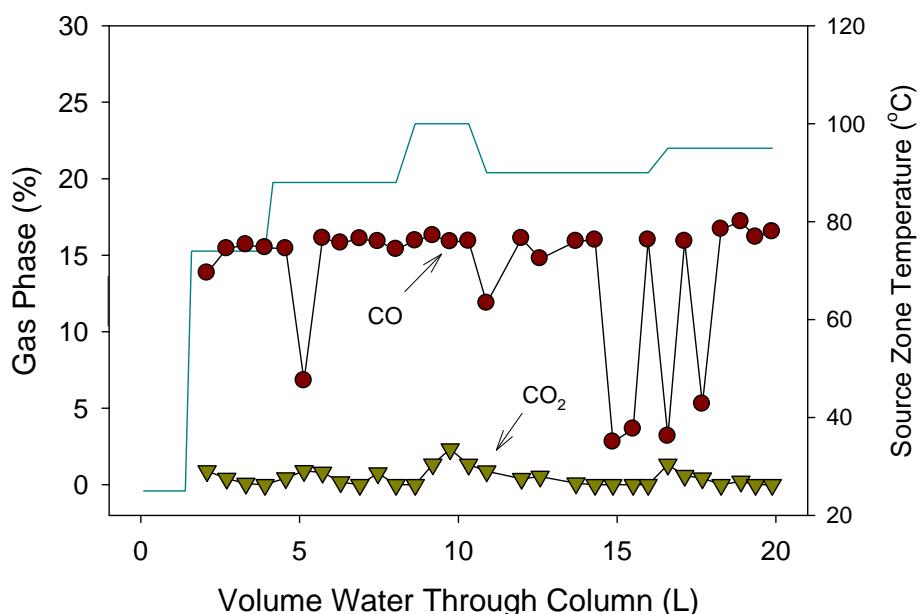
Figure 4-64. Concentration of CO and CO_2 in gas phase during ERH treatment.

In addition to aqueous phase samples, gas samples were collected from the side port located immediately down stream of the heated zone. The concentration of PCE, shown in Figure 4-64, closely tracked the concentration of PCE in aqueous phase samples. The other compounds present in the gas phase samples include hydrogen, carbon monoxide (CO), and carbon dioxide (CO_2). The concentration of CO was relatively constant over the duration of the experiment and significantly greater than for CO_2. Given that CO was not observed in batch ampule experiments with Great Lakes soil, the source of CO may have been from an interaction between the electrical current and soil within the heated zone. The slight increases in CO_2 occurred after increasing the heated zone temperature from 88 to 100 °C, and from 88 to 95 °C.

The initial mass of PCE estimated to be present in the column prior to treatment was 350 mg. The mass of PCE recovered in the aqueous phase was 313 mg and 14.2 mg remained in the soil after treatment. The amount of PCE in the gas phase was probably significant, but could not be determined since that phase was not recovered during treatment and the volume of gas within the ERH treatment cell varied with time. Thus, of the 350 mg if PCE initially present, 327.5 mg were accounted for, representing 94% of the initial PCE mass present. Thus, dechlorination of PCE may have occurred during ERH treatment of the Great Lakes soil, but it accounted for a

relatively minor fraction of the PCE mass initially present in the soil. The majority of PCE mass (89%), excluding the fraction in the gas phase, was recovered in the aqueous phase flowing through ERH treatment cell.

4.4.2. Combined ERH and Persulfate Treatment

A second 2-D ERH thermal treatment experiment was completed involving heating Great Lakes soil to 74 °C and then injecting a 32 mM sodium persulfate solution at 0.5 mL/min into the heated zone. This experiment was completed to determine if the addition of persulfate would result in the in-situ destruction of PCE and would therefore decrease the time required to remove PCE from the Great Lakes soil using thermal treatment alone.

The ERH cell was constructed with a configuration identical to that of the first column and the soil was collected from boring F3 between 8 and 10 feet bgs, the same core used in the first ERH experiment. The initial aqueous concentrations of PCE measured in the water after flowing through the Great Lakes soil prior to heating was 25 ± 3 mg/L. After passing 2 L of water or approximately 3 pore volumes through the ERH cell the temperature was increased to 74 °C and the concentration of PCE then increased to 175 mg/L, which represents a seven-fold increase in the flux of PCE (Figure 4-65). After removing 61% of the PCE mass by electrical heating, a 7 g/L persulfate solution with pH 7.0 phosphate buffer was introduced into the ERH cell that was operating at 74 °C. After introducing 100 mL of the persulfate solution, gas bubbles were observed and coincided with an increase in carbon dioxide concentrations from 0.1 to 20% (Figure 4-66). The source of carbon dioxide could have been from the oxidation of PCE or breakdown of soil carbonates as the Great Lakes soil is calcareous and has a low organic carbon content of less than 0.1%. The rate of gas bubble generation increased with subsequent addition of the persulfate solution and, in effort to avoid excessive gas formation, the temperature was decreased to 50 °C. The increase in carbon dioxide concentration was followed by an increase in chloride concentrations indicating that PCE was being dechlorinated (Figure 4-67). However, the fraction of PCE oxidized, based on the increase in chloride concentration, was less than 5% of the total mass of PCE removed from the column. The predominant fraction of mass (85%) was recovered as dissolved-phase PCE, while less than 5% of the mass remained associated with the solids in the column. Compared to the first column treated with ERH alone, the effect of persulfate was to increase PCE mass recovery from the Great Lakes soil by 43% (Figure 4-68). With ERH alone, 313 mg of PCE were recovered from 1 kg of soil at temperatures between 74 and 100 °C over a 34.8 day period, while the addition of persulfate increased the mass of PCE recovered to 447 mg at temperatures between 50 and 74 °C over 22 days. Thus, the addition of persulfate increased the mass of PCE recovered in a shorter period of time and while heating to lower temperatures. The increase in PCE recovery is hypothesized to have been caused by persulfate degrading the carbonate fraction of the Great Lakes soil in which PCE is strongly sorbed. The steel electrodes were recovered from the column after completing the experiment and did not exhibit damage or corrosion and were still operational.
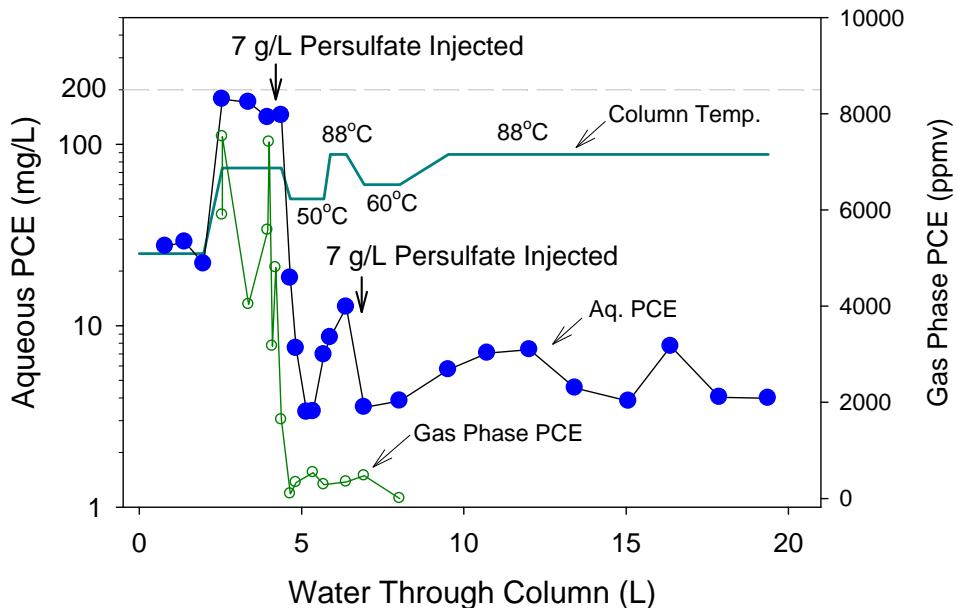
Figure 4-65. Concentration of PCE in aqueous and gas phases during ERH plus persulfate treatment.
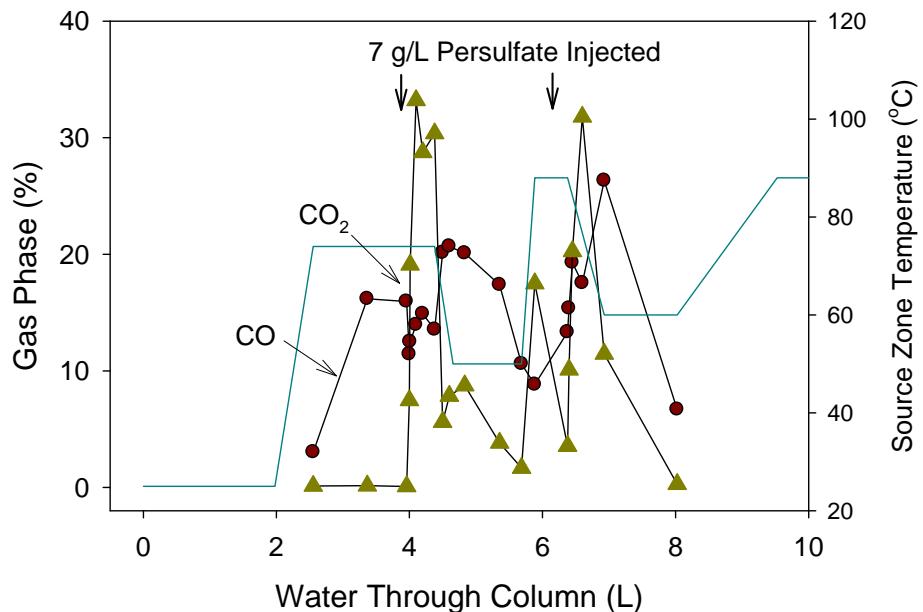
Figure 4-66. Concentration of CO and CO_2 in gas phase during ERH plus persulfate treatment.
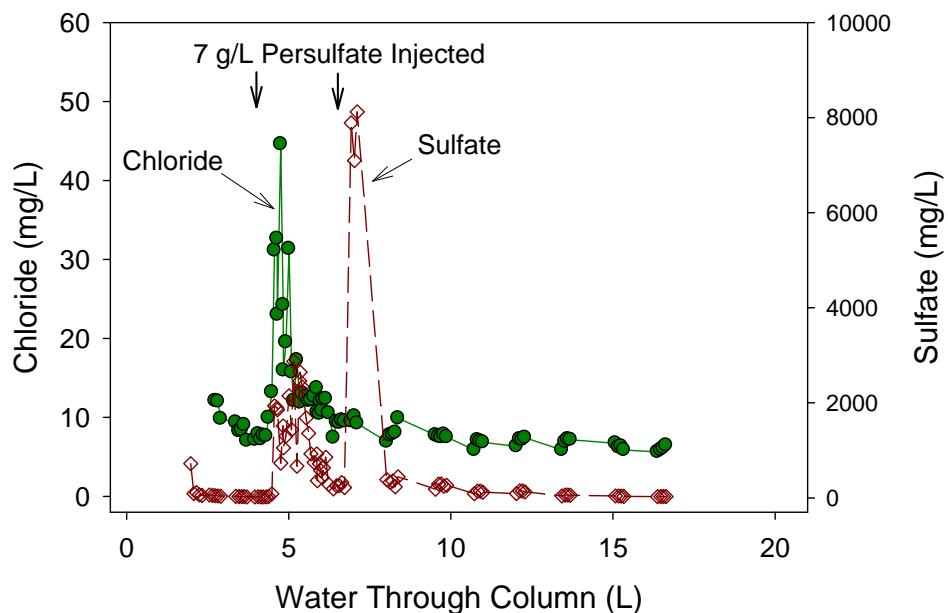
Figure 4-67. Concentration of chloride and sulfate in aqueous phase during ERH plus persulfate treatment.
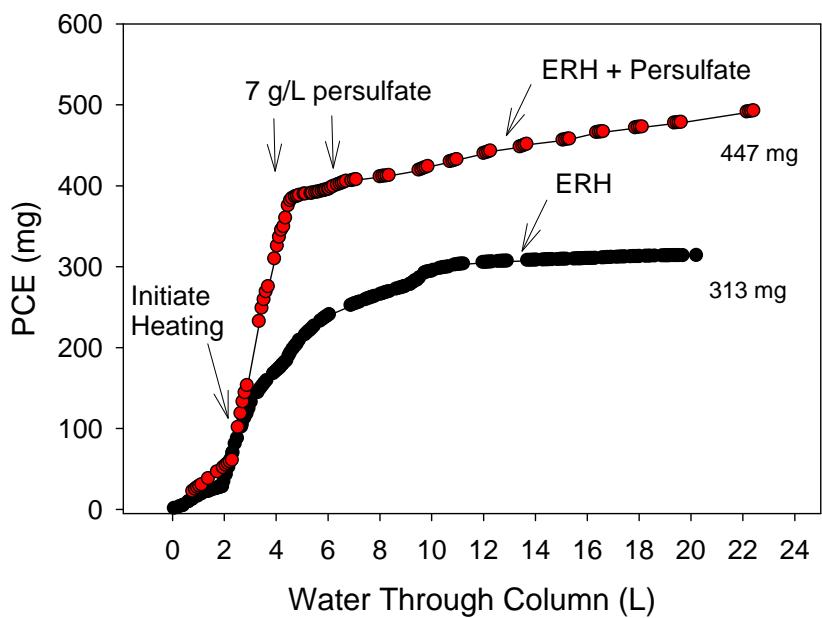
Figure 4-68. Mass of PCE recovered during ERH treatment and ERH plus persulfate treatment.

4.4.3. Combined ERH and Bioaugmentation

A third 2-D ERH thermal treatment column experiment was completed to investigate bioaugmentation of PCE-contaminated soil from the Great Lakes site during electrical heating. The purpose of this experiment was to determine if addition of a dechlorinating bacterial culture could degrade the PCE liberated from Great Lakes soil by thermal treatment without providing electron or carbon donors. The column was constructed with a configuration identical to that of the first and second columns and the soil was collected from Boring F3 between 8 and 10 feet bgs, the same core used in the first and second ERH experiments. The initial aqueous concentrations of PCE measured in the column effluent after flowing 0.68 L of water or approximately 1.4 pore volumes through the Great Lakes soil at 25 °C was 5.4 mg/L (Figure 4-69). After increasing the Great Lakes soil temperature to 74 °C, the concentration of PCE increased to 73.4 mg/L, which represents an 18 times increase in PCE mass flux from the column. The concentration of TCE was 0.3 mg/L and cis-DCE was below the detection limit of 0.1 mg/L at this point. After heating the Great Lakes soil for approximately 2.8 days and after 1.3 L of water had passed through column, 120 mL of the PCE-to-ethene-dechlorinating, methanogenic consortium OW was introduced into the uncontaminated region of the column located 10 cm down-gradient from the Great Lakes soil. The OW consortium was injected into the column region that was unheated and was maintained at a temperature of 25 °C because previous work completed had demonstrated that OW was not capable of transforming PCE to ethene at temperatures greater than 35 °C (see Section 4.3). No electron donor was provided because previous work has demonstrated that hydrogen production during heating of the Great Lakes soil. Significant levels of hydrogen gas (up to 40% by volume of gas sample) were observed during the initial two ERH column experiments completed with Great Lakes soils. Heating of the Great Lakes soil results in the production of carbon dioxide and organic acids (i.e., formate and acetate), which has the potential to fulfill the carbon source requirements of the dechlorinating bacteria.
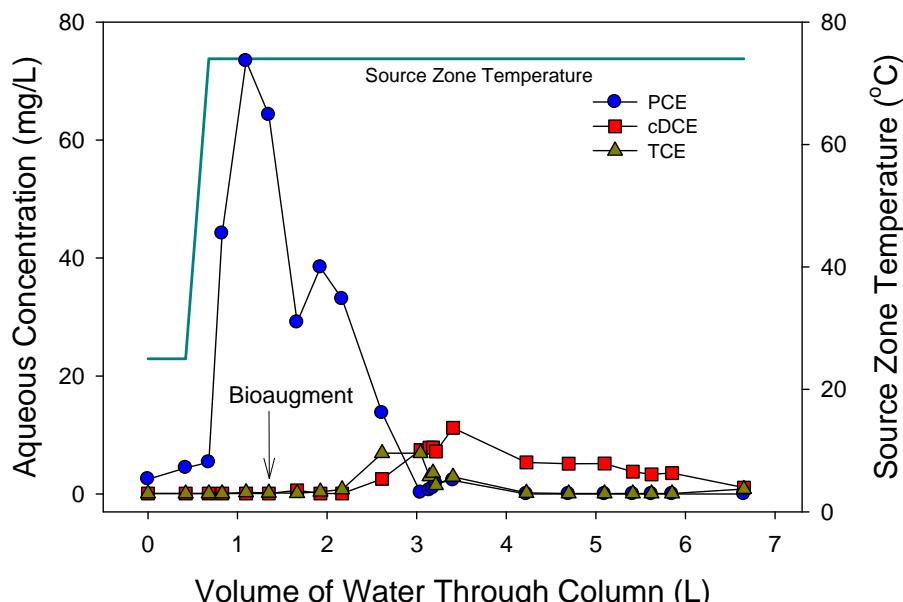
Figure 4-69. Concentrations of PCE recovered during ERH treatment and ERH plus persulfate treatment.

The PCE concentrations in the column effluent decreased to 0.2 mg/L after 3 L of water had passed through the column and the concentration of TCE and *cis*-DCE increased to 6.9 and 7.5 mg/L, respectively. Although the effluent PCE concentration was 0.2 mg/L, the concentration of PCE in the side port directly up gradient from the point of OW injection was 32.1 mg/L. The concentration of hydrogen in gas phase samples decreased from 35 to 7% and carbon dioxide decreased from 26 to 3%, while carbon monoxide levels remained constant at 16.7%. These results indicate that the OW consortium was consuming hydrogen and carbon dioxide, and reduced PCE to TCE and *cis*-DCE. After 4.2 L of water had passed through the column, the concentration of PCE and TCE were below the detection limit of 0.1 mg/L and *cis*-DCE was present at 5.2 mg/L. These levels were sustained throughout the remainder of the experiment which continued for a total of 28 days from initial bioaugmentation. Vinyl chloride and ethene were not produced 28 days after bioaugmentation, possibly because the geochemical conditions were not conducive for *Dehalococcoides* activity, or the longer column residence times are required to observe activity of organisms that dechlorinate past *cis*-DCE.

This experiment explored the potential of using heat and bioaugmentation alone to provide complete in-situ recovery and transformation of PCE to ethene. Results demonstrated that heating of Great Lakes soil produced sufficient amounts of carbon sources and electron donors (e.g., carbon dioxide, acetate, formate, hydrogen) to support microbial reductive dechlorination. This is a relevant observation suggesting that bioaugmentation alone (i.e., without the need for electron donor addition) is capable of promoting the biotic in-situ transformation of PCE to *cis*-DCE. Complete conversion of PCE to ethene was not observed over the 28-day experimental period, and may require longer residence times.

CHAPTER 5

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1. RESEARCH SUMMARY

The underlying objective of this project was to provide a more fundamental understanding of the effects of in situ thermal remediation on chlorinated ethene (PCE and TCE) phase distribution, chemical reactivity, and microbial reductive dechlorinationion. The project was structured around the following four tasks: (1) Contaminant Phase Distribution; (2) Chemical Reactivity and Byproduct Formation, (3) Microbial Reductive Dechlorination; and (4) Thermal Treatment Performance Evaluation. The research scope and general experimental approach used in each task are described below.

- Task 1 involved the collection of soil and groundwater samples from four field sites undergoing thermal remediation and determining equilibrium and kinetic desorption parameters for the field soils. Soil and groundwater samples were collected from four field sites undergoing thermal treatment; Camelot Cleaners Superfund Site (Fargo, ND), Great Lakes Naval Training Center (Great Lakes, IL), East Gate Disposal Yard (Fort Lewis, WA), and the Pemaco Superfund Site (Maywood, CA). As part of this task, temperature-dependent Henry's Law constants and aqueous solubilitiies were determined for chloroethene (PCE and TCE)-water systems over a temperature range of 20 to 90 ^{o}C.

- Task 2 involved determining the rate and extent of chloroethene degradation as a function of temperature by incubating field contaminated soils in glass ampules. Use of heat-activated sodium persulfate to increase the rate of chloroethene degradation in the field soils was also evaluated under this task. Additional experiments were performed to determine the products of incomplete combustion formed by passing gas-phase chlorinated ethenes through a quartz tube heated to fixed temperatures ranging from 20 to 800°C.

- Task 3 focused on the response and resilience of dechlorinating species and consortia to increases in temperature as well as the effect of thermal treatments on electron donor availability and the viability of competitors to dechlorinating organisms. The ability of dechlorinating consortia to control the plume of chlorinated ethenes emanating from an electrical resistive heating (ERH) treatment zone was also demonstrated under this task.

- In Task 4 laboratory-scale experiments were conducted in 1-D and 2-D flow through reactors to assess the effects of ERH treatment on contaminant mass recovery and chemical reactivity. These experiments also evaluated the use of sodium persulfate as means to enhance the in-situ destruction of chlorinated ethenes during ERH treatment.

5.2. Conclusions and Recommendations

Based on the experimental results obtained in this project, a number of important conclusions were reached regarding the abiotic reactivity, microbial reductive dechlorination, phase distribution, and recovery of chlorinated ethenes at elevated temperatures. It should be recognized that while field materials were used for these studies, the experiments were designed to investigate fundamental physical, chemical and biological processes during thermal treatment. Therefore, the conclusions described below are specific to the experimental conditions employed in each study, and should not interpreted as applicable to all thermal remediation sites or conditions. Nevertheless, these findings provide important insight into the mechanisms governing chlorinated ethene fate under representative thermal remediation scenarios.

- **Rates of Abiotic TCE and PCE Degradation are Relatively Slow at Temperatures Less than 120 ^{o}C**

Results obtained from a large matrix of ampule experiments, conducted with soil collected from four thermal treatment sites, indicate that the fraction of PCE or TCE transformed during heating is relatively small, accounting for less than 5-10% of initial contaminant mass. These findings indicate that in situ transformation processes occurring during thermal remediation are likely to provide only minimal contributions to TCE and PCE destruction at most thermal remediation sites. However, increased degradation rates and greater mass conversion may be achieved in the presence of reactive minerals or conditions during thermal treatment. For example, in ampoules containing 1% (wt.) goethite, abiotic degradation of TCE at 120 °C resulted in a half-life of 102 days over the initial 22 days of incubation.

- **Microbial Reductive Dechlorination of Native and Laboratory Pure Cultures and Consortia Ceases at Temperatures above 40 °C, but can be Recovered with Post-Treatment Bioaugmentation and Biostimulation.**

Reductive dechlorination of PCE or TCE did not occur at temperatures above 40 ºC in microcosm experiments with two PCE-to-ethene dechlorinating consortia BDI and OW, which were enriched under mesophilic conditions. Although combining bioaugmentation with or following thermal treatment requires further investigation, results obtained with the Great Lakes and Fort Lewis soils suggest that both biostimulation and bioaugmentation will be required to achieve complete and rapid dechlorination of PCE and TCE to ethene. It should be noted, however, that these results do not rule out the existence of microbes capable of PCE or TCE reductive dechlorination at elevated temperatures.

- **Formation of Byproducts During Thermal Treatment of Field Soils may Result From Degradation of Soil Organic Matter Rather than Contaminant Destruction.**

A number of potential reaction byproducts, including carbon dioxide (CO_2) and 1-butene (C_4H_8), were observed in ampules containing PCE- or TCE-contaminated field soils after incubation at elevated temperatures. However, both these compounds were detected after heating *uncontaminated* field samples, indicating that these compounds may not represent TCE and PCE degradation products. More specifically, the measured concentrations of 1-butene were similar in heated uncontaminated and contaminated soils, while concentrations of carbon dioxide were

several orders-of-magnitude higher than the amount that could be theoretically attributed to PCE degradation. Hence, the detection of elevated CO_2 levels during thermal treatment may indicate the destruction of the contaminants, but is more likely an indication of soil breakdown processes. Based on these findings, changes in CO_2 levels should not be used to infer in situ contaminant destruction or to estimate the mass of contaminant degradation.

- **Although Thermal Treatment can Increase Mass Transfer of TCE and PCE from Contaminated Soils to Groundwater, a Substantial Fraction of Contaminant Mass may Persist in Fine-Grained Soils.**

Increasing the soil temperature has been shown to decrease the sorption of chlorinated ethenes by soil, implying that thermal treatment will greatly enhance mass transfer and recovery of chlorinated ethenes from the subsurface. Even after prolonged heating (100-200 days at 95°C), however, a substantial fraction of chlorinated ethene mass was shown to be present in the solid phase, and required multiple solvent extractions to achieve complete recovery. This behavior was most apparent in Camelot Cleaners and Great Lakes field soils, both of which contain relatively high clay and silt contents. These findings demonstrate that complete mass recovery from the solid phase may require prolonged heating and extraction, or an additional treatment step. The persistence of solid-associated chlorinated ethenes is most likely to be of concern in fine-textured soils, and may contribute to contaminant rebound following thermal.

- **Use of Reactive Amendments during Thermal Treatment Leads to Enhancement in Rates of Contaminant Recovery from Slowly-Desorbing Soil Fractions.**

Results of this project indicate that the use of sodium persulfate, a water-soluble and heat-activated oxidant, in conjunction with electrical resistive heating (ERH) increased the PCE oxidation of PCE by 5% and mass recovery by 40%. Thus, addition of sodium persulfate with ERH enhanced reactivity but more importantly, increased the rate and amount of PCE mass recovery from the slowly-desorbing fraction of Great Lakes soil. The latter phenomenon was attributed to soil breakdown and the corresponding release of PCE. These findings support the consideration of reactive amendment addition at thermal remediation sites where inherent PCE or TCE reactivity is anticipated to be minimal and a large fraction of the contaminant mass is associated with the solid phase.

CHAPTER 6

REFERENCES CITED

Acinas, S.G., L.A. Marcelino, V. Klepac-Ceraj, and M.F. Polz. 2004. Divergence and redundancy of 16S rRNA sequences in genomes with multiple rrn operons. *J. Bacteriol*., 186: 2629-2635.

Aggarwal, V., H. Li, S.A. Boyd, and B.J. Teppen. 2006. Enhanced sorption of trichloroethene by smectite clay exchanged with Cs+. *Environ. Sci. Technol*., 40: 894-899.

Amos, B.K., J.A. Christ, L.M. Abriola, K.D. Pennell, and F.E. Löffler. 2007. Experimental evaluation and mathematical modeling of microbially enhanced tetrachloroethene (PCE) dissolution. *Environ. Sci. Technol*., 41: 963-970.

Amos, B.K., K.M. Ritalahti, C. Cruz-Garcia, E. Padilla-Crespo, and F.E. Löffler. 2008. Oxygen effect on *Dehalococcoides* viability and biomarker quantification. *Environ. Sci. Technol,*. 42: 5718-5726.

Anipsitakis, G.P. and D.D. Dionysiou. 2004. Radical generation by the interaction of transition metals with common oxidants. *Environ. Sci. Technol.*, 38: 3705-3712.

ASTM International. 2004. Standard Practices for Preparation of Sample Containers and for Preservation of Organic Constituents. D 3694-96. West Conshohocken, PA.

Arnold, W.A. and A.L. Roberts. 1998. Pathways of chlorinated ethylene and chlorinated acetylene reaction with Zn(0). *Environ. Sci. Technol*., 32: 3017-3025.

Arnold, W.A. and A.L. Roberts 2000. Pathways and kinetics of chlorinated ethylene and chlorinated acetylene reaction with Fe(0) particles. *Environ. Sci. Technol*., *34*: 1794-1805.

Baker, R.W. 2004. Membrane Technology and Applications. 2nd Ed., McGraw Hill, New York, NY. p. 42.

Benson, B.B. and D. Krause, Jr. 1976. Empirical laws for dilute aqueous solutions of nonpolar gases. *J. Chem. Phys.*, 64: 689-709.

Bergmann, J.G. and J. Sanik, Jr. 1957. Determination of trace amounts of chloride in naptha. *Anal. Chem.* 29: 241-243.

Buelt, J.L. and K.H. Oma. 1990. In situ heating to detoxify organic-contaminated soils. U.S. Patent No. 4,957,393.

Beyke, G., G. Smith, and V. Jurka. 2000. DNAPL remediation closure with six-phase heating. In Physical, Chemical and Thermal Technologies: Remediation of Chlorinated and Recalcitrant

Compounds, G.B. Wickramanayake and A.R. Gavaskar (Eds.), Battelle Press, Columbus, OH, pp. 183-190.

Borda, M.J., A.R. Elsetinow, D.R., Strongin, and M.A. Schoonen, M.A. 2003. A mechanism for the production of hydroxyl radical at surface defect sites on pyrite. *Geochim. Cosmochim. Ac.*, 67: 935-939.

Bridges, J.E., D. Harsh, R.H. Snow and A. Taflove. 1987. In situ decontamination of spills and landfills by radio frequency heating. U.S. Patent No. 4,670,634.

Brown, R.A., R.J. Fiacco, Jr., R.L. Lewis, and J. Dablow. 2005. Thermally based contaminant destruction. The 4th International Conference on Oxidation and Reduction Technologies for In-Situ Treatment of Soil and Groundwater, Chicago, IL. October 23-27.

Brown, R.A. 2006. Thermal activation of contaminant degradation. *In* Proceedings of the Fifth International Conference on Remediation of Chlorinated and Recalcitrant Compounds. Monterey, CA, May, Battelle Press, Columbus, OH.

Chang, Y.C., M. Hatsu, K. Jung, Y.S. Yoo, and K. Takamizawa. 2000. Isolation and characterization of a tetrachloroethylene dechlorinating bacterium, *Clostridium bifermentans* DPH-1. *J. Biosci. Bioeng*. 89: 489-491.

Chai, X.S. and J.Y. Zhu. 1998. Indirect headspace gas chromatographic method for vapor-liquid phase equilibrium study. *J. Chromatogr. A*., 799: 207-214.

Chapelle, F.H., S.K. Haack, P. Adriaens, M.A. Henry, and P.M. Bradley. 1996. Comparison of E_h and H_2 measurements for delineating redox processes in a contaminated aquifer. *Environ. Sci. Technol*., 30: 3565-3569.

Christ, J.A., C.A. Ramsburg, L.M. Abriola, K.D. Pennell, and F.E. Löffler. 2005. Coupling aggressive mass removal with microbial reductive dechlorination for remediation of DNAPL source zones: A review and assessment. *Environ. Health Perspect.,* 113 (4): 465-477.

Cline, J. D. 1969. Spectrophotometric determination of hydrogen sulfide in natural waters. *Limnol. Oceanogr*., 14: 454-458.

Conley, D.M. and C.M. Lonie. 2000. Field scale implementation of in situ thermal desorption thermal well technology. In Physical, Chemical and Thermal Technologies: Remediation of Chlorinated and Recalcitrant Compounds, G.B. Wickramanayake and A.R. Gavaskar (Eds.), Battelle Press, Columbus, OH, pp. 175-182.

Costanza, J. 2005. Degradation of tetrachloroethylene and trichloroethylene under thermal remediation conditions. Ph.D. Dissertation, Georgia Institute of Technology, Atlanta, GA.

Costanza, J., E.L. Davis, J.A. Mulholland, and K.D. Pennell. 2005. Abiotic degradation of trichloroethylene under thermal remediation conditions. *Environ. Sci. Technol.* 39: 6825-6830.

Costanza, J. and K.D. Pennell. 2006. Surface mediated abiotic degradation of chlorinated solvents at elevated temperatures. In Preprints of Extended Abstracts, 46(1): 993-999, Special Symposium on Advances in Surface-Mediated Transformation of Environmental Systems, Division of Environmental Chemistry, American Chemical Society 231st National Meeting, March 26-30, Atlanta, GA.

Costanza, J., J.A. Mulholland, K.D. Pennell, and E. Davis. 2007a. Effect of Thermal Treatments on the Chemical Reactivity of Trichloroethylene, EPA 600/R-07/091; U.S. Environmental Protection Agency, Office of Research and Development: Ada, OK.

Costanza, J. and K.D. Pennell. 2007b. Distribution and abiotic degradation of chlorinated solvents in heated field samples. *Environ. Sci. Technol.* 41:1729-1734.

Costanza, J. and K.D. Pennell. 2007c. Comparison of PCE and TCE Disappearance in Heated Volatile Organic Analysis Vials and Flame-Sealed Ampules. *Chemosphere.* doi:10.1016/j.chemosphere.2007.09.007.

Costanza, J. and K.D. Pennell. 2007d. Degradation of trichloroethylene by pyrite-goethite at elevated temperatures. *In* Preprints of Extended Abstracts, 47(1): 373-376, Special Symposium Symposia on Abiotic and Biotic Factors Affecting Contaminant Transformation at Iron Oxide Surfaces, Division of Environmental Chemistry, American Chemical Society 233rd National Meeting, March 25-29, Chicago, IL.

Costanza, J., Fletcher, K.E., Löffler, F.E., and K.D. Pennell. 2009. Fate of TCE in heated Fort Lewis soil. *Environ. Sci. Technol.,* doi**:** 10.1021/es802508x.

Costentin, C., M. Robert, and J.M. Saveant. 2003. Successive removal of chloride ions from organic polychloride pollutants. Mechanisms of reductive electrochemical elimination in aliphatic gem-polychlorides, α,β-polychloroalkenes, and α,β-polychloroalkanes in mildly protic medium. *J. Am. Chem. Soc.*, 125: 10729-10739.

Crank, J. 1975. The Mathematics of Diffusion. 2nd Ed., Oxford University Press, Oxford, UK.

Cupples, A.M, A.M. Spormann, and Perry L. McCarty. 2003. Growth of a *Dehalococcoides*-like microorganism on vinyl chloride and *cis*-dichloroethene as electron acceptors as determined by competitive PCR. *Appl. Environ. Microbiol.* 69:953-959.

Daprato, R.C., F.E. Löffler, and J.B. Hughes. 2007. Comparative analysis of three tetrachloroethene to ethene halorespiring consortia suggests functional redundancy. *Environ. Sci.Technol.*, 41: 2261-2269.

Davis, E.L. 1997. How Heat Can Enhance In-situ Soil and Aquifer Remediation: Important Chemical Properties and Guidance on Choosing the Appropriate Technology. Ground Water Issue, U.S. EPA National Risk Management Research Laboratory, Ada, OK, EPA/540/S-97/502.

Deng, Y. and J.B. Dixon. 2002. Soil organic matter and organic-mineral interactions. In Soil Mineralogy with Environmental Applications, Dixon, J.B., Schulze, D.G., Eds.; SSSA: Madison, WI.

Di, P., D.P.Y. Chang, and H.A. Dwyer. 2000. Heat and mass transfer during microwave steam treatment of contaminated soils. *ASCE J. Environ. Eng*. 126:1108-1115.

Dinicola, R.S. 2005. Hydrogeology and Ttrichloroethene Contamination in the Sea-Level Aquifer Beneath the Logistic Center, Fort Lewis, Washington. U.S. Geological Survey, Reston, VA.; Scientific Investigations Report 2005-5035.

Duhamel, M., S. D. Wehr, L. Wu, H. Rizvi, D. Seepersad, S. Dworatzek, E. A. Cox, and E. A. Edwards. 2002. Comparison of anaerobic dechlorinating enrichment cultures maintained on tetrachloroethene, trichloroethene, *cis*-dichloroethene and vinyl chloride. *Water Research* 36:4193-4202.

Ellis, D. E., E. J. Lutz, J. M. Odom, R. J. Buchanan, Jr., C. L. Barlett, A. Lee, M. R. Harkness, and K. A. DeWeerd. 2000. Bioaugmentation for accelerated in situ anaerobic bioremediation. *Environ. Sci. Technol.* 34:2254-2260.

Federal Remediation Technology Roundtable (FRTR). 2002. Section 4.10 Thermal Treatment. Remediation Technologies Screening Matrix and Reference Guide, Version 4.0. Available at: www.frtr.gov/matrix2.

Fletcher, K.E., K.M. Ritalahti, K.D. Pennell, K. Takamizawa, and F. E.Löffler. 2008. Resolution of culture *Clostridium bifermentans* DPH-1 into two populations, a *Clostridium* sp. and tetrachloroethene-dechlorinating *Desulfitobacterium hafniense* strain JH1. *Appl. Environ. Microbiol.,* 74: 6141-6143.

Friis, A.K., H.J. Albrechtsen, G. Heron, and P. L. Bjerg. 2005. Redox processes and release of organic matter after thermal treatment of a TCE-Contaminated aquifer. *Environ. Sci. Technol.*, 39: 5787-5795.

Friis, A.K., H.J. Albrechtsen, E. Cox, and P. L. Bjerg. 2006. The need for bioaugmentation after thermal treatment of a TCE-contaminated aquifer: Laboratory experiments. *J. Contam. Hydrol.,* 88: 235-248.

Friis, A.K., E.A. Edwards, H.-J. Albrechtsen, K.S. Udell, M. Duhamel, and P.L. Bjerg. 2007. Dechlorination after thermal treatment of a TCE-contaminated aquifer: Laboratory experiments. *Chemosphere*, *67*, 816-825.

Gerdes, K.S., S. Kaslusky and K.S. Udell. 1998. Containment of downward migration of DNAPLs during steam enhanced extraction. *In* Nonaqueous-Phase Liquids: Remediation of Chlorinated and Recalcitrant Compounds, G.B. Wickramanayake and R.E. Hinchee (Eds.), Battelle Press, Columbus, OH, pp. 31-36.

Gerritse, J., V. Renard, T.M.P. Gomes, P.A. Lawson, M.D. Collins, and J.C. Gottschal. 1996. *Desulfitobacterium* sp. strain PCE1, an anaerobic bacterium that can grow by reductive dechlorination of tetrachloroethene or ortho-chlorinated phenols. *Arch. Microbiol.*, 165: 132-140.

Gossett, J. 1987. Measurement of Henry's Law constants for C_1 and C_2 chlorinated hydrocarbons. *Environ. Sci. Technol.*, 21: 202-208

Goulden, P.D. and D.H.J. Anthony. 1978. Kinetics of uncatalyzed peroxydisulfate oxidation of organic materials in fresh water. *Anal. Chem.*, 50: 953-958.

Griffin, B.M, J.M. Tiedje, and F.E. Löffler. 2004. Anaerobic microbial reductive dechlorination of tetrachloroethene to predominately *trans*-1,2-dichloroethene. *Environ. Sci. Technol.*, 38: 4300-4303.

Haas, J.R. and E.L. Shock. 1999. Halocarbons in the environment: Estimates of thermodynamic properties for aqueous chloroethylene species and their stabilities in natural settings. *Geochim. Cosmochim. Ac.*, 63: 3429-3441.

Haddad, P.R. and A.L. Heckenberg. 1988. Trace determinination of sulfide by reversed-phase ion-interaction chromatography using pre-column derivitization. *J. Chromatogr.*, 447(2): 415-420.

Haselow, J.S., R.L. Siegrist, M. Crimi, and T. Jarosch. 2003. Estimating the total oxidant demand for in situ chemical oxidation design. *Remediation,* 13(4): 5-16.

He, J.Z., Y. Sung, M.E. Dollhopf, B. Z. Fathepure, J.M. Tiedje, and F.E. Löffler. 2002. Acetate versus hydrogen as direct electron donors to stimulate the microbial reductive dechlorination process at chloroethene-contaminated sites. *Environ. Sci. Technol.*, 36: 3945-3952.

He, J., K.M. Ritalahti, M.R. Aiello, and F.E. Löffler. 2003a. Complete detoxification of vinyl chloride by an anaerobic enrichment culture and identification of the reductively dechlorinating population as a *Dehalococcoides* species. *Appl. Environ. Microbiol*. 69:996-1003.

He, J.Z., K.M. Ritalahti, K.L. Yang, S.S. Koenigsberg, and F. E. Löffler. 2003b. Detoxification of vinyl chloride to ethene coupled to growth of an anaerobic bacterium. *Nature*, 424: 62-65.

He, J.Z., Y. Sung, R. Krajmalnik-Brown, K.M. Ritalahti, and F.E. Löffler. 2005. Isolation and characterization of *Dehalococcoides* sp. strain FL2, a trichloroethene (TCE)-and 1,2-dichloroethene-respiring anaerobe. *Environ. Microbiol.*, 7: 1442-1450.

Heath, W.O., R.L. Richardson, and S.C. Goheen. 1994. Heating solid earthen material, measuring moisture and resistivity. U.S. Patent No. 5,330,291.

Heron, G., T.H. Christensen, and C.G. Enfield. 1998a. Henry's law constant for trichloroethylene between 10 and 95°C. *Environ. Sci. Technol*. 32:1433-1437.

Heron, G., M. Van Zutphen, T.H. Christensen, and C.G. Enfield. 1998b. Soil heating for enhanced remediation of chlorinated solvents: A laboratory study on resistive heating and vapor extraction in a silty, low-permeable soil contaminated with trichloroethylene. *Environ. Sci. Technol.* 32:1474-1481.

Heron, G., T.H. Christensen, T. Heron, and T.H. Larsen. 1998c. Thermally enhanced remediation at DNAPL sites: The competition between downward mobilization and upward volatilization. *In* Nonaqueous-Phase Liquids: Remediation of Chlorinated and Recalcitrant Compounds, G.B. Wickramanayake and R.E. Hinchee (Eds.), Battelle Press, Columbus, OH, pp. 193-198.

Holliger, C., D. Hahn, H. Harmsen, W. Ludwig, W. Schumacher, B. Tindall, F. Vazquez, N. Weiss, and A.J.B. Zehnder. 1998. *Dehalobacter restrictus* gen. nov. and sp. nov., a strictly anaerobic bacterium that reductively dechlorinates tetra- and trichloroethene in an anaerobic respiration. *Arch. Microbiol.*, 169: 313-321.

Iben, I.E.T., W.A. Edelstein, R.B. Sheldon, A.P. Shapiro, E.E. Uzgiris, C.R. Scatena, S.R. Blaha, W.B. Silverstein, C.R. Brown, G.L. Stegemeier, and H.J. Vinegar. 1996. Thermal blanket for *in-situ* remediation of surfacial contamination: A pilot test. *Environ. Sci. Technol.* 30:3144-3160.

Jackson, M.L. 2005. Soil Chemical Analysis: Advanced Course. Revised 2nd edition. Parallel Press, University of Wisconsin-Madison Libraries, Madison, WI, p. 44.

Jeffers, P.M., L.M. Ward, L.M. Woytowitch, and N.L. Wolfe. 1989. Homogeneous hydrolysis rate constants for selected chlorinated methanes, ethanes, ethenes and propanes. *Environ. Sci. Technol.*, 23: 965-969

Johnson, L.A. Jr. and B.C. Suddeth. 1989. Contained Recovery of Oil Waste. U.S. Patent No. 4,884,460.

Kalinski, R.J. and W.E. Kelly. 1993. Estimating water content of soils from electrical resistivity. *ASTM Geotech. Testing J.* 16:323-327.

Kaslusky, S.F. and K.S. Udell. 2002. A theoretical model of air and steam co-injection to prevent downward migration of DNAPLs during steam-enhanced extraction. *J. Contam. Hydrol.*, 55: 213-232.

Kolb, B. and L.S. Ettre. 2006. Static Headspace-Gas Chromatography, Theory and Practice. 2nd Ed., John Wiley&Sons, Inc., Hobokem, NJ. p. 233-235.

Knauss, K.G., M.J. Dibley, R.N. Leif, D.A. Mew, and R.A. Aines. 1999. Aqueous oxidation of trichloroethene (TCE): A kinetic analysis. *Appl. Geochem.*, 14: 531-541.

Knauss, K.G., M.J. Dibley, R.N. Leif, D.A. Mew, and R.A. Aines. 2000. The aqueous solubility of trichloroethene (TCE) and tetrachloroethene (PCE) as a function of temperature. *Appl. Geochem.*, 15: 501-512.

Kovacs, D.A., and D.H. Kampbell. 1999. Improved method for the storage of groundwater samples containing volatile organic analytes. *Arch. Environ. Con. Tox.,* 36: 242-247. Krajmalnik-Brown, R., T. Hölscher, I.N. Thomson, F.M. Saunders, K.M. Ritalahti, and F.E. Löffler. 2004. Genetic identification of a putative vinyl chloride reductase in *Dehalococcoides* sp. strain BAV1. *Appl. Environ. Microbiol.,* 70: 6347-6351.

Krauter, P., D. MacQueen, and D. Bishop. 2005. Effects on Subsurface Electrical Heating and Steam Injection on the Indigenous Microbial Community, UCRL-JC-122299, Lawrence Livermore National Laboratory, Palo Alto, CA.

Kwan, W.P. and B.M. Voelker. 2003. Rates of hydroxyl radical generation and organic compound oxidation in mineral-catalyzed fenton-like systems. *Environ. Sci. Technol.*, 37: 1150-1158.

Lanthier, M., R. Villemur, F. Lépine, J.G. Bissaillon, and R. Beaudet. 2001. Geographic distribution of *Desulfitobacterium frappieri* PCP-1 and *Desulfitobacterium* spp. in soils from the province of Quebec, Canada. *FEMS Microbiol. Ecol.*, 36: 185-191.

Ledvay, J.M., F.E. Löffler, M. Dollhopf, M.R. Aiello, G. Daniels, B.Z. Fathepure, M. Gebhard, R. Heine, R. Helton, J. Shi, R. Krajmalnik-Brown, C.L. Major, Jr., M.J. Barcelona, E. Petrovskis, R. Hickey, J.M. Tiedje, and P. Adriaens. 2003. Bioreactive barriers: a comparison of bioaugmentation and biostimulation for chlorinated solvent remediation. *Environ. Sci. Technol.* 37:1422-1431.

Leif, R.N., M. Chiarappa, R.D. Aines, R.L. Newmark, K.G. Knauss and C. Eaker. 1998. In situ hydrothermal oxidative destruction of DNAPLs in a creosote contaminated site. *In* Physical, Chemical and Thermal Technologies: Remediation of Chlorinated and Recalcitrant Compounds, G.B. Wickramanayake and R.E. Hinchee (Eds.), Battelle Press, Columbus, OH, pp. 133-138.

Liang, C., Z.-S. Wang, and C.J. Bruell. 2007. Influence of pH on persulfate oxidation of TCE at ambient temperatures. *Chemosphere,* 66: 106-113.

Linstrom, P.J and W.G. Mallard, Editors. 2005. **NIST Chemistry WebBook, NIST Standard Reference Database Number 69.** National Institute of Standards and Technology, Gaithersburg MD, 20899, June. Available at: http://webbook.nist.gov.

Loeppert, R.H. and D.L. Suarez. 1996. Carbonate and gypsum. In Sparks, D.L.; Page, A.L.; Helmke, P.A.; Loeppert, R.H.; Soltanpour, P.N.; Tabatabai, M.A.; Johnston, C.T.; Sumner, M.E. (Eds.) In *Methods of Soil Analysis*. Part 3. SSSA Book Ser. 5. SSSA, Madison, WI, p. 451-455.

Löffler, F.E., R.A. Sanford, and K.M. Ritalahti. 2005. Enrichment, cultivation, and detection of reductively dechlorinating bacteria. *Methods Enzymol.*, 397: 77-111.

Magnuson, J.K., M.F. Romine, D.R. Burris, and M.T. Kingsley. 2000. Trichloroethene reductive dehalogenase from *Dehalococcoides ethenogenes*: sequence of *tceA* and substrate range characterization. *Appl. Environ. Microbiol.*, 66: 5141-5147.

Major, D. W., M. L. McMaster, E. E. Cox, E. A. Edwards, S. M. Dworatzek, E. R. Hendrickson, M. G. Starr, J. A. Payn, and L. W. Buonamici. 2002. Field demonstration of successful bioaugmentation to achieve dechlorination of tetrachloroethene to ethene. *Environ. Sci. Technol.* 36:5106-5116.

Maymó-Gatell, X., Y.-T., Chien, J. M. Gossett, and S. H. Zinder. 1997. Isolation of a bacterium that reductively dechlorinates tetrachloroethene to ethene. *Science* 276:1568-1571.

Mechati, F., E. Roth, V. Renault, V. Risoul, G. Trouve, and P. Gilot. 2004. Pilot scale and theoretical study of thermal remediation of soils. *Environ. Eng. Sci.*, 21: 361-370.

Mulholland, J.A., M. Lu and D-H. Kim. 2000. Pyrolytic growth of polycyclic aromatic hydrocarbons by cyclopentadienyl moieties. Proc. Combust. Inst. 28: 2593-2599.

Müller, J.A., B.M. Rosner, G. von Abendroth, G. Meshluham-Simon, P.L. McCarty, and A.M. Spormann. 2004. Molecular identification of the catabolic vinyl chloride reductase from *Dehalococcoides* sp. strain VS and its environmental distribution. *Appl. Environ. Microbiol.*, 70: 4880-4888.

National Research Council (NRC), 2005. Contaminants in the Subsurface: Source Zone Assessment and Remediation. National Academy Press. Washington, DC.

Naval Facilities Engineering Service Center (NFESC). 2007. Final Report – Cost and Performance Review of Electrical Resistance Heating (ERH) for Source Treatment, Technical Report TR-2279-Env; Naval Facilities Engineering Command, Port Hueneme, CA.

Newmark, R.L. and R.D. Aines. 1995. Summary of the LLNL Gasoline Spill Demonstration - Dynamic Underground Stripping Project, Lawrence Livermore National Laboratory, Berkeley Environmental Restoration Center, University of California, Berkeley.

Okeke, B.C., Y.C. Chang, M. Hatsu, T. Suzuki, and K. Takamizawa. 2001. Purification, cloning, and sequencing of an enzyme mediating the reductive dechlorination of tetrachloroethylene (PCE) from *Clostridium bifermentans* DPH-1. *Can. J. Microbiol.*, 47: 448-456.

Parker, L.V. and S.A. Ranney. 1994. Effect of concentration on sorption of dissolved organics by PVC, PTFE, and stainless steel well casings. *Ground Water Monit. Remed.*, Summer, 139-149.

Parsons, T.R., Y. Maita, and C.M. Lalli. 1984. A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamon Press, Oxford.

Richardson, R.E., C.A. James, V.K. Bhupathiraju, and L. Alvarez-Cohen. 2002. Microbial activity in soils following steam treatment. *Biodegradation*, 13: 285-295.
Ritalahti, K.M. and F.E. Löffler. 2004. Populations implicated in anaerobic reductive dechlorination of 1,2-dichloropropane in highly enriched bacterial communities. *Appl. Environ. Microbiol.*, 70: 4088-4095.

Ritalahti, K.M., F.E. Löffler, E.E. Rasch, and S.S. Koeningsberg. 2005. Bioaugmentation for chlorinated ethene detoxification: bioaugmentation and molecular diagnostics in the bioremediation of chlorinated ethene-contaminated sites. *Ind. Biotechnol.*, 1: 114-118.

Ritalahti, K.M., B.K. Amos, Y. Sung, Q. Wu, S.S. Koenigsberg, and F.E. Löffler. 2006. Quantitative PCR targeting 16S rRNA and reductive dehalogenase genes simultaneously monitors multiple *Dehalococcoides* strain. *Appl. Environ. Microbiol.*, 72: 2765-2774.

Robain, H., C. Camerlynck, G. Bellier, and A. Tabbagh. 2003. Laboratory measurement of electrical resistivity versus water content on small soil cores. *Geophys. Res. Abstr.* 5:

Ryan, T.A., C. Ryan, E.A. Seddon, and K.R. Seddon. 1996. *Phosgene and Related Carbonyl Chlorides*. Monograph 24, Topics in Inorganic and General Chemistry. R.J.H. Clark, Editor. Elsevier Science, B.V., Amsterdam, The Netherlands. p. 398.

Schmidt, R., J. Gudbjerg, T.O. Sonnenburg, and K.H. Jensen. 2002. Removal of NAPLs from the unsaturated zone using steam: Prevention of downward migration by injecting mixtures of steam and air. *J. Contam. Hydrol.*, 55: 233-260.

Scholz-Muramatsu, H., A. Neumann, M. Messmer, E. Moore, and G. Diekert. 1995. Isolation and characterization of *Dehalospirillum multivorans* gen-nov, sp-nov, a tetrachloroethene-utilizing, strictly anaerobic bacterium. *Arch. Microbiol.*, 163: 48-56.

Schumacher, B.A., Minnich, M.M., Zimmerman, J.H., Blasdell, J., 2000. Integrity of VOA-Vial Seals. EPA/600/R-00/066. United States Environmental Protection Agency, Solid Waste and Emergency Response, Washington, DC.

She, H.Y. and B.E. Sleep. 1999. Removal of perchloroethylene from a layered soil system by steam flushing. *Ground Wat. Monitor. Remed.*, 19: 70-77.

Shelby, J.E., 1974. Helium deuterium, and neon migration in a common borosilicate glass. *J. Appl. Phys.,* 45: 2146-2149.

Shimizu, T., S. Ohshima, K. Ohtani, K. Hoshino, K. Honjo, H. Hayashi, and T. Shimizu. 2001. Sequence heterogeneity of the ten rRNA operons in *Clostridium perfringens*. Syst. *Appl. Microbiol.*, 24: 149-156.

Shimotori, T. and W.A. Arnold. 2002. Henry's Law constants of chlorinated ethylenes in aqueous alcohol solutions: Measurement, estimation, and thermodynamic analysis. *J. Chem. Eng. Data.*, 47: 183-190.

Staudinger, J. and P.V. Roberts. 2001. A critical compilation of Henry's law constant temperature dependence relations for organic compounds in dilute aqueous solutions. *Chemosphere,* 44: 561-576.

Stegemeier, G.L. and H.J. Vinegar. 2001. Thermal conduction heating for in-situ thermal desorption of soils. *In* Hazardous and Radioactive Waste Treatment Technologies Handbook. C.H. Oh (Ed.), CRC Press, Boca Raton, FL, Chapter 4.6.

Stookey, L.L. 1970. Ferrozine - A new spectrophotometric reagent for iron. *Anal. Chem.* 42: 7, 779.

Stroo, H.F., M. Unger, C.H. Ward, M.C. Kavanaugh, C. Vogel, A. Leeson, J.A. Marqusee, and B.P. Smith. 2003. Remediating chlorinated solvent source zones. *Environ. Sci. Technol.*, 37: 225A-230A.

Stumm, W. and J.J. Morgan. 1996. Aquatic Chemistry; 3rd Edition, John Wiley and Sons, Inc., New York, NY, p.691.

Sung, Y., K.M. Ritalahti, R.A. Sanford, J.W. Urbance, S.J. Flynn, J.M. Tiedje, and F.E. Löffler. 2003. Characterization of two tetrachloroethene-reducing, acetate-oxidizing anaerobic bacteria and their description as *Desulfuromonas michiganensis* sp. nov. *Appl. Environ. Microbiol.*, 69: 2964-2974.

Sung, Y., K.E. Fletcher, K.M. Ritalahti, R.P. Apkarian, N. Ramos-Hernández, R.A. Sanford, N. M. Mesbah, and F.E. Löffler. 2006a. *Geobacter lovleyi* sp. nov. strain SZ, a novel metal-reducing and tetrachloroethene-dechlorinating bacterium. *Appl. Environ. Microbiol.*, 72: 2775-2782.

Sung, Y., K.M. Ritalahti, R.P. Apkarian, and F.E. Löffler. 2006b. Quantitative PCR confirms purity of strain GT, a novel trichloroethene-to-ethene-respiring *Dehalococcoides* isolate. *Appl. Environ. Microbiol.*, 72: 1980-1987.

Suyama, A., M. Yamashita, S. Yoshino, and K. Furukawa. 2002. Molecular characterization of the *pceA* reductive dehalogenase of *Desulfitobacterium* sp. strain Y51. J. *Bacteriol.*, 184: 3419-3425.

Suyama, A., R. Iwakiri, K. Kai, T. Tokunaga, N. Sera, and K. Furukawa. 2001. Isolation and characterization of *Desulfitobacterium* sp. strain Y51 capable of efficient dehalogenation of tetrachloroethene and polychloroethanes. *Biosci. Biotech. Bioch.*, 65: 1474-1481.

Truex, M., T. Powell, and K. Lynch, 2007. In situ dechlorination of TCE during aquifer heating. *Ground Water Monit. Remed.,* 27: 96-105.

Udell, K.S. 1997. Thermally enhanced removal of liquid hydrocarbon contaminants from soils and groundwater. In Subsurface Restoration, Ward, C.H., Cherry, J.A., and Scalf, M.R. (eds.), Ann Arbor Press, Chelsea, MI. p. 251-270.

Udell, K.S., J.R. Hunt, N. Sitar and L.D. Steward, Jr. 1991. Process for In-Situ Decontamination of Subsurface Soil and Ground Water. U.S. Patent No. 5,018,576.

Udell, K.S., M. Itamura, L. Alvarez-Cohen, and M. Hernandez. 1994. NAS Lemoore JP-5 Cleanup Demonstration, Berkeley Environmental Restoration Center, University of California, Berkeley, CA.

Udell, K.S. and M.T. Itamura. 1998. Removal of dissolved solvents from heated heterogeneous soils during depressurization. In Physical, Chemical, and Thermal Technologies: Remediation of Chlorinate and Recalcitrant Compounds, Wickramanayake, G.B., Hinchee, R.E., Eds., Battelle Press, Columbus, OH, pp. 57-62.

U.S. Department of Energy (DOE). 2000. Hydrous pyrolysis oxidation/dynamic underground stripping. Innovative Technology Summary Report, DOE/EM-0504.

U.S. Environmental Protection Agency (EPA). 1995a. IITRI Radio Frequency Heating Technology. Site Technology Capsule, U.S. EPA, Washington, DC, EPA/540/R-94/527a.

U.S. Environmental Protection Agency (EPA). 1995b. KAI Radio Frequency Heating Technology. Site Technology Capsule, U.S. EPA, Washington, DC, EPA/540/R-94/528a.

U.S. Environmental Protection Agency (EPA). 1995c. Method 552.2: Determination of haloacetic acids and dalapon in drinking water by liquid-liquid extraction, derivatization, and gas chromatography with electron capture detector, Revision 1.0. In Methods for the Determination of Organic Compounds in Drinking Water, EPA/600/R-95/131, U. S. Environmental Protection Agency, Office of Research and Development, Washington, DC, Supplement III, p. 1-33.

U.S. Environmental Protection Agency (EPA). 2000. Western Research Institute Contained Recovery of Oily Wastes (CROW) Process. Innovative Technology Evaluation Report, U.S. EPA, Washington, DC. EPA/540/R-00/500

U.S. Environmental Protection Agency (EPA). 2004. In Situ Thermal Treatment of Chlorinated Solvents: Fundamentals and Field Applications, EPA 542-R-04-010, U. S. Environmental Protection Agency, Office of Superfund Remediation and Technology Innovation, Washington, DC, p. 1-34.

U.S. Environmental Protection Agency (EPA). 2008. An Introduction to Indoor Air Quality. http://www.epa.gov/iaq/co.html.

Vane, L.M. and E.L. Giroux. 2000. Henry's Law constants and micellar partitioning of volatile organic compounds in surfactant solutions. *J. Chem. Eng. Data.*, 45: 38-47.

Villemur, R., M. Lanthier, R. Beaudet, and F. Lépine. 2006. The *Desulfitobacterium* genus. *FEMS Microbiol*. Rev., 30: 706-733.

Vinegar, H.J., G.L. Stegemeier, E.P. de Rouffignac, and C.C. Chou. 1993. Vacuum Method for Removing Soil Contaminants Utilizing Thermal Conductive Heating. U.S. Patent No. 5,190,405.

Viollier, E., P.W. Inglett, K. Hunter 2000. The ferrozine method revisited: Fe(II)/Fe(III) determination in natural waters. Appl. Geochem., 15(6):785-790.

Volek, C.W. and J.A. Pryor. 1972. Steam distillation drive: Brea Field, California. *J. Pet. Tech.* 24:899-906.

Waldemer, R.H., P.G. Tratnyek, R.L. Johnson, and J.T. Nurmi, 2007. Oxidation of Chlorinated Ethenes by Heat-Activated Persulfate: Kinetics and Products. *Environ. Sci. Technol.*, 41: 1010-1015.

Wicks, G.G., R.L. Schulz and D.E. Clark. 1999. Microwave technology for waste management applications including disposition of electronic circuitry. In Environmental Issues and Waste Management Technologies in the Ceramic and Nuclear Industries IV, J.C. Marra and G.T. Chandler (Eds.), 93:89-96.

Wilson, D.J. and A.N. Clarke. 1994. Soil vapor stripping. In Hazardous Waste Site Soil Remediation, D.J. Clarke and A.N. Clarke (Eds.), Marcel Dekker, NY, Chapter 4.

Wu, Q., R.A. Sanford, and F.E. Löffler. 2006. Uranium(VI) reduction by *Anaeromyxobacter dehalogenans* strain 2CP-C. *Appl. Environ. Microbiol.*, 72: 3608-3614.

Yang, Y., J.A. Mulholland and U. Akki. 1998. Formation of furans by gas-phase reactions of chlorophenols. 27th Symposium on Combustion, Combustion Institute, pp. 1761-1768.

Zinder, S.H. and J.M. Gossett. 1995. Reductive dechlorination of tetrachloroethene by a high rate anaerobic microbial consortium. *Environ. Health Perspect.,* 103(5): 5-7.

APPENDIX A

TECHNICAL CONTRIBUTIONS

A.1. JOURNAL PUBLICATIONS (REVERSE CHRONOLOGICAL ORDER)

Fletcher, K.E., J. Costanza, K.D. Pennell, and F.E. Löffler. 2009. Availability of electron donors for reductive dechlorination following thermal treatment. (*in preparation for ES&T*)

Fletcher, K.E., N.S. Ramaswamy, K.D. Pennell, and F.E. Löffler. 2009. Vinyl chloride accumulation during reductive dechlorination at elevated temperatures. *(in preparation for AEM).*

Costanza, J., E.L. Davis, J.A. Mulholland, and K.D. Pennell. 2009. Reactivity of TCE under thermal conductive remedation conditions. (*in preparation for submission to ES&T*).

Costanza, J., G. Otaño, J. Callaghan, and K.D. Pennell. 2009. PCE oxidation by persulfate in solids containing reactors. (*in preparation for submission to ES&T*).

Costanza, J., K.E. Fletcher, F.E. Löffler, and K.D. Pennell. 2009. Fate of trichloroethene in heated Fort Lewis soil. *Environmental Science and Technology* 43: doi:10.1021/es802508x.

Fletcher, K.E., K.M. Ritalahti, K.D. Pennell, K. Takamizawa, and F.E. Löffler. 2008. Resolution of culture *Clostridium bifermentans* DPH-1 into two populations, a *Clostridium* sp. and tetrachloroethene-dechlorinating *Desulfitobacterium hafniense* strain JH1. *Applied and Environmental Microbiology*, 74: 6141-6143.

Costanza, J. and K.D. Pennell. 2008. Comparison of PCE and TCE disappearance in heated volatile organic analysis vials and flame-sealed ampules. *Chemosphere,* 70: 2060-2067.

Costanza, J. and K.D. Pennell. 2007. Distribution and abiotic degradation of chlorinated solvents in heated field samples. *Environmental Science and Technology,* 41(5):1729-1734.

A.2. CONFERENCE PROCEEDINGS AND ABSTRACTS

Pennell, K.D., J. Costanza, F.E. Löffler, K.E. Fletcher, N. Ramaswamy, G. Otaño, and J. Callaghan. 2008. Fate of chlorinated ethenes during thermal treatment. SERDP Partners in Environmental Technology Technical Symposium & Workshop, December 3-6, 2008, Washington, DC.

Pennell, K.D., J. Costanza, F.E. Löffler, K.E. Fletcher, N. Ramaswamy, G. Otaño, J. Callaghan. 2008. Reactivity of chlorinated ethenes during thermal treatment. Sixth International Conference on Remediation of Chlorinated and Recalcitrant Compounds, May 19-22, 2008, Monterey, CA.

Costanza, J. G. Otaño, J. Callaghan, K.D. Pennell. 2008. Combining ERH and ISCO for treatment of PCE-contaminated soils. Sixth International Conference on Remediation of Chlorinated and Recalcitrant Compounds, May 19-22, 2008, Monterey, CA.

Fletcher, K.E., J. Costanza, K.D. Pennell, and F.E. Löffler. 2008. Availability of electron donors for reductive dechlorination following thermal treatment. Sixth International Conference on Remediation of Chlorinated and Recalcitrant Compounds, May 19-22, 2008, Monterey, CA.

Pennell, K.D., F.E. Löffler, J. Callaghan, G. Otaño, K.E. Fletcher, J. Costanza. Combining ERH and ISCO for treatment of PCE-contaminated soils. SERDP Partners in Environmental Technology Technical Symposium & Workshop, December 3-6, 2007, Washington, DC.

Costanza, J., G. Otaño, J. Callaghan, and K.D. Pennell. Evaluation of combined persulfate and thermal treatment to degrade residual PCE in soil. The 5th International Conference on Oxidation and Reduction Technologies for In-Situ Treatment of Soil and Groundwater, September 24-27, 2007, Niagara Falls, NY.

Costanza, J. and K.D. Pennell. 2007. Degradation of trichloroethylene by pyrite-goethite at elevated temperatures. *In* Preprints of Extended Abstracts, 47(1): 373-376, Special Symposium Symposia on Abiotic and Biotic Factors Affecting Contaminant Transformation at Iron Oxide Surfaces, Division of Environmental Chemistry, American Chemical Society 233rd National Meeting, March 25-29, 2007, Chicago, IL.

Costanza, J., K.D. Pennell, F.E. Löffler, and K.E. Fletcher. 2006. Fate of TCE in Fort Lewis Soils under thermal remediation conditions: Sorption and biotic degradation. Partners in Environmental Technology Technical Symposium & Workshop, November 28-30, 2006 Washington, DC.

Costanza, J. and K.D. Pennell. 2006. Degradation of chlorinated solvents during in-situ thermal treatment to be presented at the 58th Southeast Regional Meeting of the American Chemical Society, November 1-4, 2006 Augusta, GA.

Costanza, J. and K.D. Pennell. 2006. Thermal enhanced recovery and abiotic degradation of chlorinated solvents in contaminated field samples. *In* Preprints of Extended Abstracts, 46(2): 1199-1204, Graduate Student Awards Symposium, Division of Environmental Chemistry, American Chemical Society 232nd National Meetings, Sept. 10-14, 2006, San Francisco, CA (*invited*).

Costanza, J., K.D. Pennell, and B.M. Jorgensen. 2006. Feasibility of tetracholoethylene degradation during Six-Phase Heating™ of Camelot Cleaners Superfund Site. Remediation of Chlorinated and Recalcitrant Compounds: The Fifth International Conference, May 22-26, 2006 Monterey, CA.

Costanza, J., K.D. Pennell, J.A. Mulholland., and E.L. Davis. 2006. Degradation of trichloroethylene under high-temperature thermal source zone removal conditions. Remediation of Chlorinated and Recalcitrant Compounds: The Fifth International Conference, May 22-26, 2006 Monterey, CA.

Costanza, J. and K.D. Pennell. 2006. Surface mediated abiotic degradation of chlorinated solvents at elevated temperatures. Advances in Surface-Mediated Transformation of Environmental Systems, Symposia Papers Presented Before the Division of Environmental Chemistry, American Chemical Society, 231st National ACS Meeting, March 26-30, 2006 Atlanta, GA.

Costanza, J., K.D. Pennell, F.E. Löffler, K.E. Fletcher, E.L. Davis, and K.P Lynch. 2005. Degradation of trichloroethylene under thermal remediation conditions. Partners in Environmental Technology Technical Symposium & Workshop, November 28-30, 2005 Washington, DC.

Costanza, J., K.D. Pennell, and B.M. Jorgensen. 2005. Abiotic degradation of chlorinated ethenes under thermal remediation conditions. The 4th International Conference on Oxidation and Reduction Technologies for *In-Situ* Treatment of Soil and Groundwater, October 24, 2005 Chicago, IL.

A.3. TECHNICAL REPORTS

Costanza, J. and K.D. Pennell. 2005. *Incubation of Samples from the Camelot Cleaners Site, West Fargo, ND*. Current Environmental Solutions (CES), Kennewick, WA.

A.4. HONORS AND AWARDS

Kelly Fletcher received a Student Presentation Award at the 2009 RemTEC Summit for her platform presentation, "Stable Carbon Isotope Fractionation of 1,2-Dichloropropane During Degradation by *Dehalococcoides* Populations."

John Callaghan received an Undergraduate Research Internship Award (PURA) in Spring 2007

Jed Costanza received the 2006 Graduate Student Paper Award from the Division of Environmental Chemistry, American Chemical Society for his paper, "Thermal Enhanced Recovery and Abiotic Degradation of Chlorinated Solvents in Contaminated Field Samples."

Jed Costanza received the 2006 Georgia Tech Sigma Xi Best Ph.D. Thesis Award for his dissertation, "Degradation of Tetrachloroethylene and Trichloroethylene under Thermal Remediation Conditions."

Kelly Fletcher received a National Science Foundation Graduate Research Fellowship Program Award (NSF GRFP), Spring 2005.

A.5. STUDENT SUPPORT

Ms. Kelly Fletcher, Ph.D. candidate
   Dissertation Title: Chlorinated Solvent and Uranium Bioremediation: Identifying Organisms of Interest, Assessing Activity, and Enhancing Treatment through Coupled Approaches

Ms. Gretell Otano, M.S. CE (May 2008)
   SRP Title: Activated Persulfate Treatment of TCE and PCE
   Current Position: Environmental Engineer, Union Carbide Corp.

Ms. Nivi Ramaswamy, M.S. EnvE (May 2007)
   SRP Title: Bioremediation of Chlorinated Ethenes During Thermal Remediation
   Current Position: Environmental Engineer, Geosyntec Consultants, Acton, MA

Mr. John Callaghan, B.S. ChBE (May 2007)
   Project: Heat-Activated Chemical Reactivity of TCE
   Current Position: Chemical Engineer, Albemarle Corp.

 Dr. Jed Costanza, Ph.D. (December 2005)
   Dissertation Title: Effects of In-Situ Heating on the Chemical Reactivity and Byproduct Formation of DNAPLs
   Current Position: USEPA, Washington, DC